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ON THE BIOLOGY AND RELATIONSHIPS OF THE LAMELLIBRANCH *XYLOPHAGA DORSALIS* (TURTON)*

By R. Denison Purchon, Ph.D.
University of Bristol

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(Text-figs. 1–16)

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INTRODUCTION

Xylophaga dorsalis (Turton) is a lamellibranch specialized for boring into timber in the sea. It occurs chiefly in floating timber, and probably for this reason and because of its much smaller economic importance it has never been studied in the same detail as many species of *Teredo* which do great damage to wooden ships and pier piles. The relationships of the genus *Xylophaga* are also obscure. Although it never bores into stone it has hitherto been included in the Pholadidae together with *Pholas* and other genera which are exclusively rock borers.

Specimens of *Xylophaga dorsalis* were obtained from Plymouth, and others, in two heavily-colonized pieces of driftwood, from Cullercoats and from Millport. The anatomy of the species has been studied in detail since it has never as yet been adequately described. The ciliary mechanisms in the mantle cavity were examined under the binocular microscope after the application of powdered carmine and of fine carborundum powder. Duboscq Brasil was found to be the most suitable general fixative. Sections were cut at thicknesses of from 3 to 8μ and were stained in Delafield's haematoxylin and erythrosin.

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* Owing to Dr R. D. Purchon's absence from this country on active service, this paper, which represents the greater part of the thesis he presented for the degree of Doctor of Philosophy, has been prepared for publication by Prof. C. M. Yonge.

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In addition the ciliary currents and ctenidia of *Teredo megotara* Hanley and *T. norvegica* Spengler, the latter obtained from Port Erin and from Millport, were examined. This was done primarily for comparative purposes and to determine the effect of the modification of the feeding mechanisms due to the wood-boring habit upon the ciliary currents in the mantle cavity. These mechanisms have never previously been studied and new observations are recorded.

Work on *T. megotara* was carried out at the Marine Biological Station, Plymouth, in August 1937. The author wishes to express his gratitude to Dr S. Kemp, F.R.S., and members of the staff at Plymouth for the facilities provided. The remainder of the work was conducted in the Department of Zoology, University of Bristol, at the suggestion and under the direction of Prof. C. M. Yonge.

HABITAT AND HABITS OF *XYLOPHAGA DORSALIS*

Xylophaga dorsalis resembles members of the Teredinidae in its habit of boring into timber and not into stone. It is typically found in driftwood and only occasionally in fixed structures such as dock gates. It is usually, although not always, present in wood uninfected by *Teredo*. The animal which damages submarine telegraph cables and is known to cable repairers as "Teredo" is actually *Xylophaga*. Its ability to live in the gutta-percha sheath of these cables indicates that it cannot be completely dependent on wood as a source of nutriment. This matter will be discussed later.

The shell of a fully-grown *X. dorsalis* is of much the same size as that of *Teredo norvegica* the greatest antero-posterior dimension being about 10 mm. But, unlike the latter, it almost completely encloses the animal. Owing to the globular shape of the animal the borings of *Xylophaga dorsalis* are much less extensive than those of the Teredinidae. They are usually 2–3 cm. long and open to the exterior by a small pore, unless the surface wood has been broken away. Shell valves have been obtained from driftwood with an antero-posterior length of slightly over 1 in. These are probably shells of *X. praestans*. The accessory plates differ markedly in shape from those of *X. dorsalis*, and to each pair is attached a large transparent horny median plate. No such structure has been recorded in *X. dorsalis*. The excavations made by these large specimens were several inches long (Fig. 1*a*).

Xylophaga does not line its burrow with a calcareous deposition as do *Teredo* and *Bankia*, nor does it possess pallets with which to close the opening of the burrow.

Being globular in shape it might be supposed that when *Xylophaga* releases its attachment by means of the foot to the forward end of the burrow it would roll about in the cavity. But this cannot occur, nor can the animal withdraw far from the boring end of the burrow, because the posterior end of the burrow is tightly packed with faecal pellets.

As shown in Fig. 2*a*, the exhalant siphon differs from that of other lamellibranchs in that it opens at the base of the siphonal process, on its dorsal side close to the posterior margins of the shell valves (S). The opening, which is oval, entire and devoid of tentacles, lies well within the burrow (ES). The faecal pellets are not expelled with sufficient force to carry them out of the burrow and so accumulate at its posterior end as a compact mass, consolidated with mucus, which surrounds the siphonal process through which the exhalant water current passes. The presence of this faecal accumulation prevents any extensive movements of the animal.

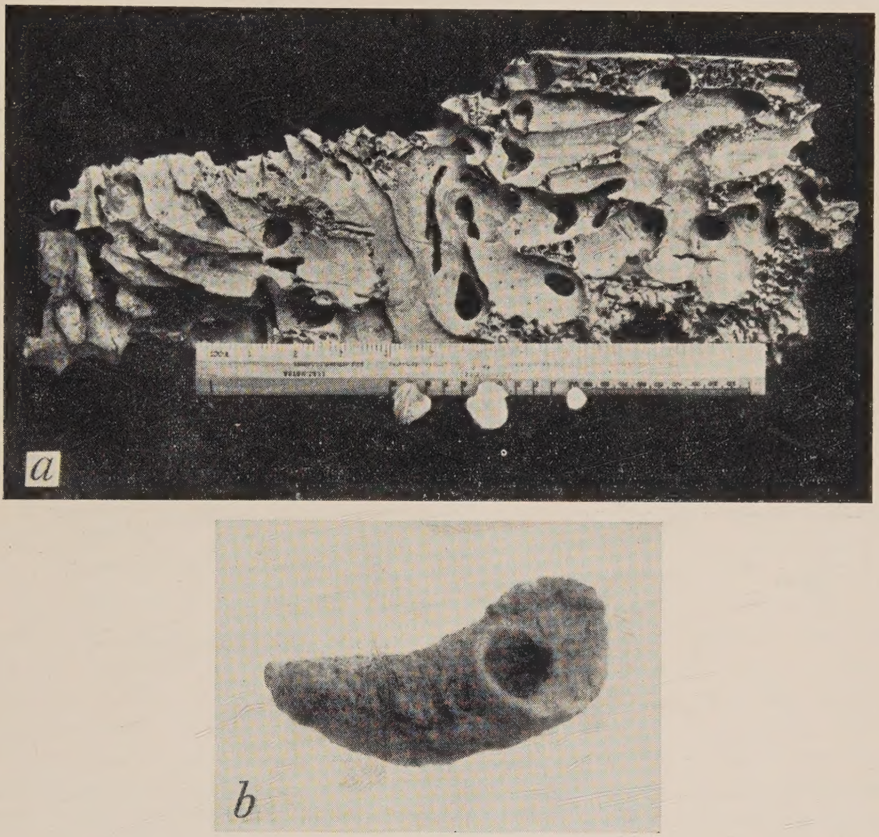

Fig. 1. *a*, Piece of wood bored by large specimens of *Xylophaga*, probably *X. praestans*. This sample was obtained by Messrs Neale and West, Trawler owners of Cardiff, and is now in an exhibition case in the Technical College, Cardiff. *b*, Faecal concretion taken from the posterior end of a burrow of one of the large specimens of *Xylophaga*.

The inhalant siphon (Fig. 2*a*, IS) is elongate, tubular and delicate. It may extend for as much as 1 cm. from the surface of the wood. The circular aperture is fringed with about six delicate tentacles (Figs. 2*a*, 6*b*, IS).

It is often possible to tell almost at a glance whether wood is colonized by *Teredo* or by *Xylophaga*, as in the former the siphons arise in pairs and in the latter they arise singly, being exclusively inhalant. The dorsal surface of the inhalant siphon possesses a pair of longitudinal lappets which are crenate for the proximal third of their length and then bear fine tentacles (Figs. 2*a*, 6*b*). They probably represent the original lateral walls of an elongated exhalant siphon which was attached throughout most of its length to the inhalant siphon, but the orifice of which has migrated backwards to the position which it now occupies. These fimbriated lappets support the sides of the cavity through the

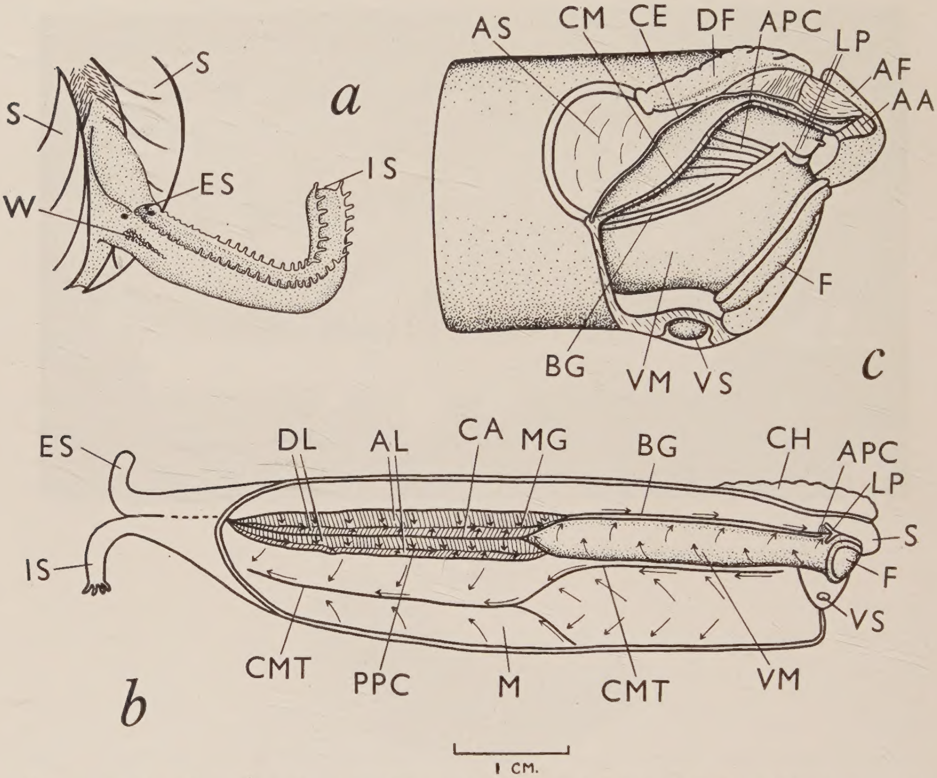

Fig. 2. *a*, *Xylophaga dorsalis*: posterior end of the shell valves and the siphonal process: ES, exhalant siphon; IS, inhalant siphon; S, shell; W, wedge-shaped mass of mucous glands. *b*, *Teredo norvegica*: mantle cavity opened laterally to show the course of the ciliary currents: AL, ascending lamella of ctenidium; APC, anterior portion of ctenidium; BG, branchial groove; CA, ctenidial axis; CH, cephalic hood; CMT, ciliated mantle tract; DL, descending lamella of ctenidium; ES, exhalant siphon; F, foot; IS, inhalant siphon; LP, labial palps; M, mantle; MG, marginal groove; PPC, posterior portion of ctenidium; VM, visceral mass; VS, ventral articulation of shell: other lettering as before. *c*, *Teredo navalis* (after Lazier): anterior portion of the mantle cavity opened to show the structure of the anterior portion of the ctenidium and the labial palps: AA, anterior adductor; AF, anterior pallial fold; AS, auricle of shell; CE, cut edge of shell; CM, cut edge of mantle; DF, dorsal pallial fold. Other lettering as before.

faecal mass along which the exhalant water current passes. The dried remains of the siphonal process in a faecal concretion from the large specimens which were probably *X. praestans* suggest that in this species the siphonal process is similarly modified (Fig. 1*b*).

It is curious to observe that, although both Adams (1853–8) and Jeffries (1865) examined *Xylophaga* in sufficient detail to record the two longitudinal pectinated ridges which ornament the dorsal surface of the siphonal process, they erred in stating that the siphonal process was divided at its distal end into distinct inhalant and exhalant tubes. A similar error was made by Forbes & Hanley (1853) and by Pelseneer (1906), and erroneous illustrations are given by the first three of these authors. Forbes & Hanley, however, were made aware of their mistake by a Mr Cocks, as is recorded in Volume II of their work (pp. 375–6).

Near the base of the siphonal process a pair of opaque white spots can often be seen, one on each side of the opening of the exhalant siphon (Fig. 2*a*). Below each of these lies a thin but conspicuous wedge of mucous glands which have an opaque bubbly appearance (Figs. 2*a*, 6*b*, w). These may act as organs of temporary attachment (*Teredo* is weakly attached to the calcareous lining of its burrow at the base of the siphonal process), or they may exude mucus which assists in consolidating the faecal mass in the posterior end of the burrow.

Water expelled from the exhalant siphon passes slowly through the burrow and disperses at its mouth. Water is collected by the inhalant siphon about 1 cm. from the opening of the burrow and so a supply of fresh water is ensured.

It is difficult to observe the way in which *Teredo* clings to the end of its burrow, because when the burrow is opened the animal retreats from the boring end. Miller (1924), however, succeeded in observing *Teredo* making boring movements by dissecting open the burrow and covering the opening by a thin strip of glass which was stuck down at the edges by vaseline. After a short time the animal expanded and returned to the boring end of the burrow. Miller observed boring operations by means of a binocular microscope before the animal had covered the strip of glass with a deposition of calcium carbonate.

Unlike *Teredo*, however, *Xylophaga* maintains its grip on the end of the burrow while the latter is being opened, and there is no difficulty in observing the mode of attachment. Suction doubtless plays an important part in the attachment of the foot, but it was frequently observed that when a specimen of *X. dorsalis* was being removed from its burrow with the foot firmly attached, this could be prised slowly from the wood by a needle. Attachment of the foot thus continues after suction can no longer be maintained. There appears to be a sticky secretion which assists attachment. The adhesive surface of the foot is roughly circular and is bounded by a conspicuous ridge. Median to this ridge, the surface of the foot bears two lateral crescentic opaque patches one on each side; in the centre of the foot the surface is not opaque. The ridge which surrounds the adhesive surface of the foot bears a conspicuous opaque raised disk in a median dorsal position (Fig. 6*b*, OF). Serial sections of the foot show that mucoid glands are present close to the epidermis in the regions which appear opaque externally. These glands are probably derived from the byssus apparatus, and presumably it is a sticky secretion from these glands which assists in fastening the foot to the boring end of the burrow. Sections through the foot of *Teredo* show that it also possesses mucoid glands which may assist in attachment.

Ciliary Mechanisms in the Mantle Cavities of *Teredo* and *Xylophaga*

Teredo norvegica Spengler

The ctenidia.

Sigerfoos (1908) has shown that, in the post-larval development of *Bankia* (*Xylotrya*) *gouldi* Bartsch, the ctenidium, which is composed of only one demibranch, becomes separated into two portions of unequal size due to the great broadening of the tenth or eleventh filament. The ctenidium is also divided into two portions in *Teredo*, the number of filaments in the anterior portion (Fig. 2*b*, APC) varying in different species. In *T. navalis* L. (Lazier, 1924) there are five anterior gill filaments (Fig. 2*c*, APC). In *Teredo*, as in *Bankia*, only one demibranch is present on each side of the animal. Ridewood (1903) and also Atkins (1937*b*) considered this to be the inner demibranch; but it has been shown elsewhere (Purchon, 1939) that in *Teredo* and in *Xylophaga* it is the *outer demibranch* which remains.

The ciliary mechanisms in the mantle cavity of *Teredo* have been compared with those of the closely related rock-borers in the family Pholadidae, of which American species in the genera *Pholadidea*, *Zirphaea* and *Barnea* were examined by Kellogg (1915). In the Pholadidae the ctenidia are each composed of *two complete demibranchs* (Kellogg, 1915).

Both Ridewood (1903) and Sigerfoos (1908) considered that in *Teredo* and *Bankia* the anterior portion of the gill consisted of *descending* filaments. The afferent branchial vein travels in the ctenidial axis and, bearing this in mind, consideration of the figures given by Sigerfoos (1908) for *Bankia* makes it evident that in the anterior portion of the gill the descending lamella is represented by the inner wall of the branchial groove, and that the filaments present actually form the *ascending lamella* of the gill. The same holds good for *Teredo*.

The anterior portion of the ctenidium lies close to the labial palps at the sides of the foot (Figs. 2*b*, 4, APC). The posterior part is situated for the most part posterior to the visceral mass (Fig. 2*b*, PPC). These two portions of the gill are connected by the branchial groove (Fig. 2*b*, BG), which is an extension of the marginal food groove (Fig. 2*b*, MG). In *T. norvegica*, as in *T. navalis* (see Ridewood, 1903), owing to the ventral position of the gill axis, the descending lamellae pass outwards almost horizontally (Fig. 3*g*) instead of downwards

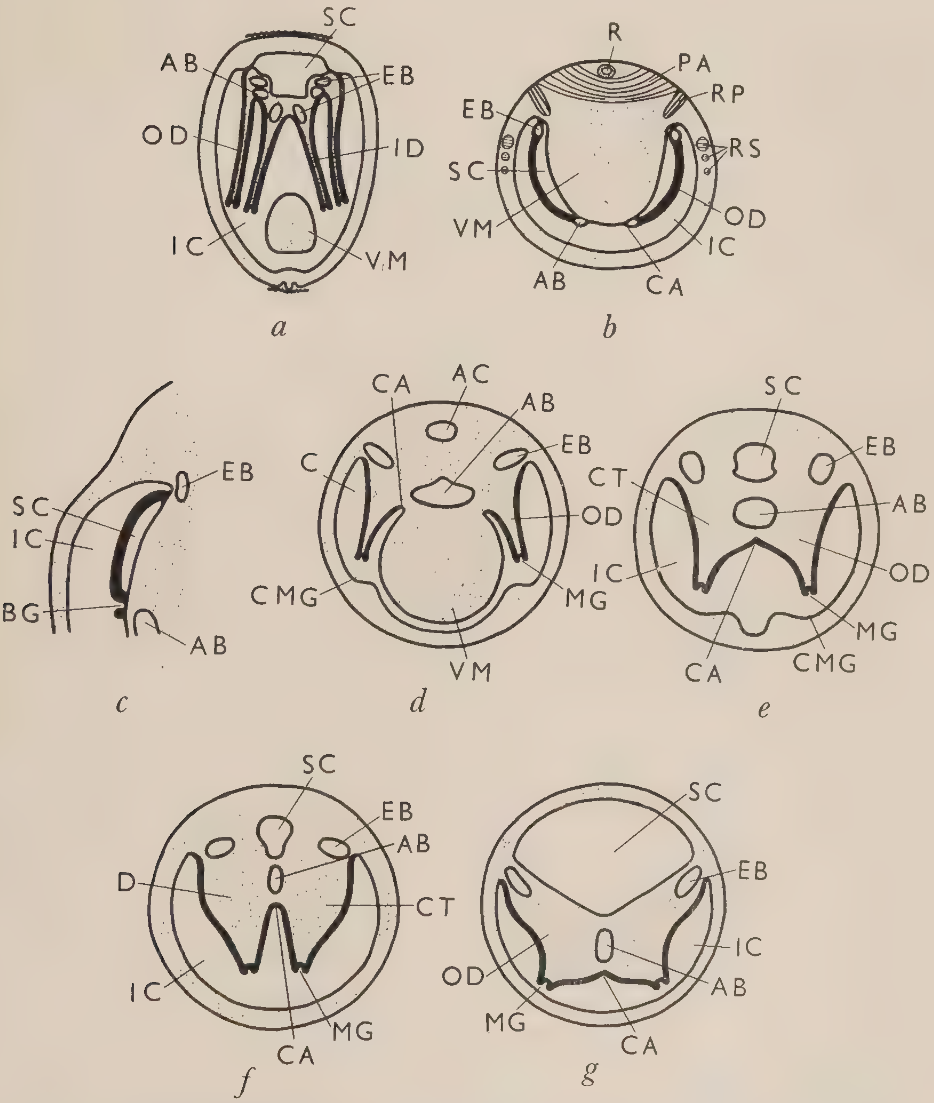
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Fig. 3. Diagrammatic transverse sections through the soft parts of various rock-boring and wood-boring lamellibranchs.

a, *Barnea parva*.
b, *Xylophaga dorsalis*.
c, *Teredo norvegica*, through the anterior portion of the ctenidium.
d, *Bankia gouldi* (after Sigerfoos), through the posterior end of the visceral mass.
e, *B. gouldi* (after Sigerfoos), through the posterior portion of the ctenidium.
f, *Teredo megotara*, through the posterior portion of the ctenidium.
g, *T. navalis* (after Ridewood), through the posterior portion of the ctenidium.

AB, afferent branchial vein; AC, anal canal; CMG, ciliated mantle groove; CT, ctenidium; EB, efferent branchial vein; IC, infra-branchial cavity; ID, inner demibranch; OD, outer demibranch; PA, posterior adductor; R, rectum; RP, retractor pedis muscle; RS, retractor muscles of siphonal process; SC, supra-branchial cavity. Other lettering as before.

as in *Bankia gouldi* (Fig. 3, *d*, *e*) and in *Teredo megotara* (Fig. 3*f*). In *T. norvegica* the anterior portion of the ctenidium is composed of ten ascending filaments, of which the tenth is shorter than the others in the specimens examined. Occasionally one of the filaments may be broader near the marginal groove than elsewhere as is the case in filament two (Fig. 4).

The ctenidial axes are situated in a dorso-lateral position opposite the visceral mass, but near the posterior end of the visceral mass they approach the median line, where they fuse, as do the afferent branchial vessels. The posterior portions of the ctenidia thus lie side by side and their descending lamellae arise from a common axis (Fig. 3 *e*, *f*, *g*, CA). The ctenidia are flat and homorhabdic; upon both portions of the ctenidium the cilia beat downwards into the marginal groove. In the marginal food grooves and in the branchial groove the cilia beat forwards, and all particles which enter them are driven towards the base of the labial palps (Figs. 2*b*, 4).

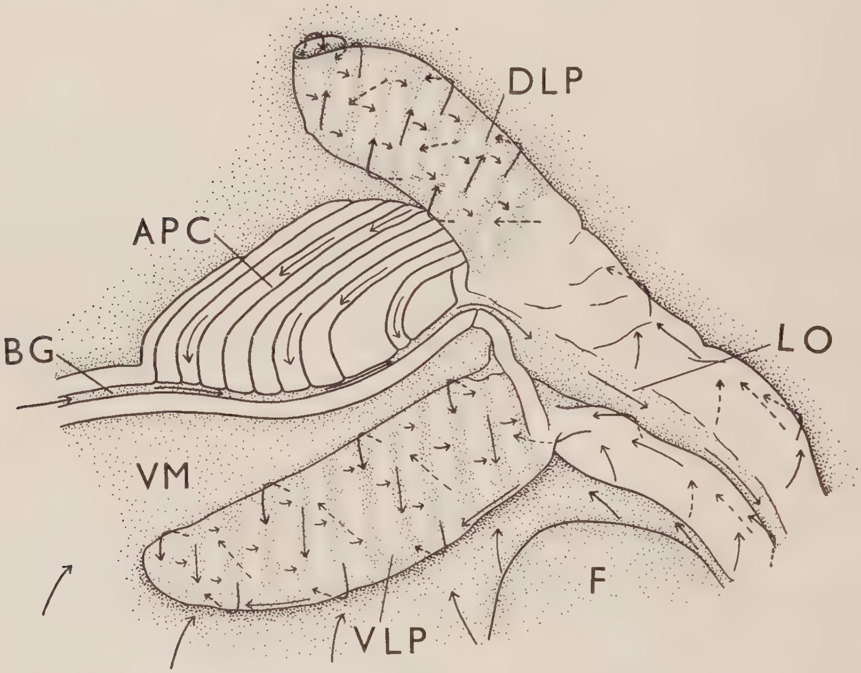


Fig. 4. *Teredo norvegica*: anterior portion of the ctenidium and the labial palps: DLP, dorsal labial palp; LO, lateral oral groove; VLP, ventral labial palp. Other lettering as before.

As summarized by Atkins (1936, 1937*a*) there are two main types of ctenidial sorting mechanisms in the Lamellibranchia whereby large particles are rejected and small particles are passed to the mouth. In the first of these, large particles pass down the lamellae along the crests of the plicae into the marginal groove, whence they are eventually rejected; small particles, however, pass upwards in the grooves of the plicae and forwards along the ctenidial axes, or along the upper borders of the ascending lamellae, towards the mouth (Arcidae, Anomiidae, *Pecten*, *Ostrea*, etc.). In the second type both large and small particles pass down the ctenidium, but the small particles are allowed to pass forwards in the marginal food groove, while large particles are prevented —in some forms—by the presence of fan-like groups of large guarding cilia (*Pinna*, *Thracia*, *Musculus*, *Montacuta*, etc.).

Close examination of the ctenidia of *Teredo norvegica* showed that no such sorting mechanisms occur; all particles placed on the ctenidia passed down into the marginal food groove whence they are all passed forwards to the base of the labial palps (Fig. 2*b*, LP; Fig. 4, DLP, VLP). There is no ciliary current in the longitudinal direction either along the ctenidial axis or along the dorsal borders of the ascending lamellae.

The labial palps.

Deshayes (1845–8) and Quatrefages (1849) probably worked upon *T. norvegica*; they both figured animals with long strap-shaped labial palps, and *T. norvegica* is the only species in European waters which answers to such a description.

Of the species under consideration, the labial palps of *T. norvegica* exhibit the least reduction in size and complexity; those of *Bankia gouldi* are, according to Sigerfoos (1908), reduced to small ridges on the sides of a slight groove approaching the mouth. In *Teredo navalis* (Fig. 2*c*, LP) Lazier found "that the dorsal palps are small and inconspicuous but quite distinct, whilst the ventral palps are reduced to slightly raised ciliated patches". The labial palps of *T. megotara* are also greatly reduced; they will be described in due course.

The labial palps of *T. norvegica* are relatively large strap-shaped organs which hang freely in the mantle cavity just in front of the anterior portion of the ctenidium (Fig. 4). In comparison with the labial palps of a typical lamellibranch they are greatly reduced, although they are the largest yet recorded in the Teredinidae. Their outer surfaces are smooth, and their inner, opposed, surfaces possesses eleven indistinct transverse ridges (Fig. 4). The ciliary mechanisms on the palps are complex, and can best be understood by close reference to the figure (Fig. 4). On the inner surfaces the current in the grooves is directed towards the lower edge and over this on to the outer surface. Along the free lower edge the current is towards the tip of the palp and away from the mouth. On the transverse ridges the cilia beat across the palp towards the mouth. On the outer surface the cilia beat obliquely backwards and upwards towards the free upper edge of the palp and over this on to the inner surface as shown by broken arrows in Fig. 4.

When extremely small quantities of carmine particles are applied to the palps, the grooves between the ridges become occluded. The particles are therefore carried by the cilia on the ridges towards the mouth. When a little more carmine is added the grooves open and the ridges become less distinct. The most prominent ciliary activity is now that in the transverse grooves. Material passed forwards from the branchial groove is picked up by the palps and passed down one of the transverse grooves on to the outer surface. Here it passes obliquely upwards and backwards on to the inner surface again. A large mass of carmine quickly forms, and this circles the palp and slowly moves backwards to the tip of the palp where it is transferred to the mantle and passed backwards in a rejection current to the base of the inhalant siphon. The labial palps therefore exercise quantitative selection, and only few and fine particles are allowed to reach the mouth.

The walls of the lateral oral groove are well defined and possess powerful rejection currents. Small quantities of carmine travel down the centre of the groove to the mouth; but if a suspension of carmine is added here, it is seen that on the walls of the lateral oral groove ciliary currents pass downwards and backwards on both surfaces. Those on the inner surfaces pass over on to the outer surfaces and thence on to the outer surface of the labial palps.

The visceral mass and the mantle.

Lazier (1924) found that in *T. navalis* the ciliation of the mantle was restricted to two narrow strips opposite the marginal and branchial grooves. In *T. norvegica* the ciliation of the mantle can be divided into two distinct types. The first consists of groups of cilia scattered over the major part of the visceral mass and the mantle. The second consists of a pair of ciliated mantle tracts (Fig. 2*b*, CMT) which in the anterior region of the mantle cavity lie close to the branchial grooves. As shown in Fig. 2*b*, they approach the mid-ventral line opposite the posterior end of the visceral mass and thence pass backwards as a single tract to the base of the inhalant siphon. Throughout these tracts the cilia beat backwards.

As shown in Fig. 2*b*, the scattered groups of cilia on the mantle beat towards the ciliated mantle tracts and assist in keeping the surface of the mantle clean. The cilia on the mantle tracts beat powerfully, and by their action skeins of mucus are drawn from the general surface of the mantle and incorporated in the stream passing backwards to the base of the inhalant siphon. This action tends to mask the activity of the scattered groups of cilia.

In the vicinity of the labial palps the scattered cilia on the visceral mass beat forwards and particles borne in these currents are collected by the palps (Fig. 4). This is probably for the collection of stray wood fragments formed during boring operations. The cilia on the remainder of the visceral mass beat towards the branchial grooves, though it is probable that under normal conditions material collected by these cilia is passed to the ciliated mantle tracts (Fig. 2*b*).

The surface of the visceral mass and of the mantle which bears the scattered groups of cilia appears to consist of a shallow syncytial epithelium. This bears groups of long cilia which arise from distinct basal granules arranged in a plate close to the surface (Fig. 5*a*). The junction between each basal granule and its cilium can be distinguished, as also can a series of fine fibres passing inwards in the form of a spindle from the basal granules to the base of the syncytium. No lateral cell walls can be distinguished, but a certain rather regular patchiness of the cytoplasm is suggestive of cell demarcation.

Near the anterior gill filaments, this syncytium gradually becomes deeper, and lateral cell walls can be distinguished, and each cell in this region possesses a group of long cilia. This suggests that the great elongation of the visceral mass and of the mantle has been accompanied by a stretching of the epithelial layers as well as by a proliferation of the cells comprising the epithelium.

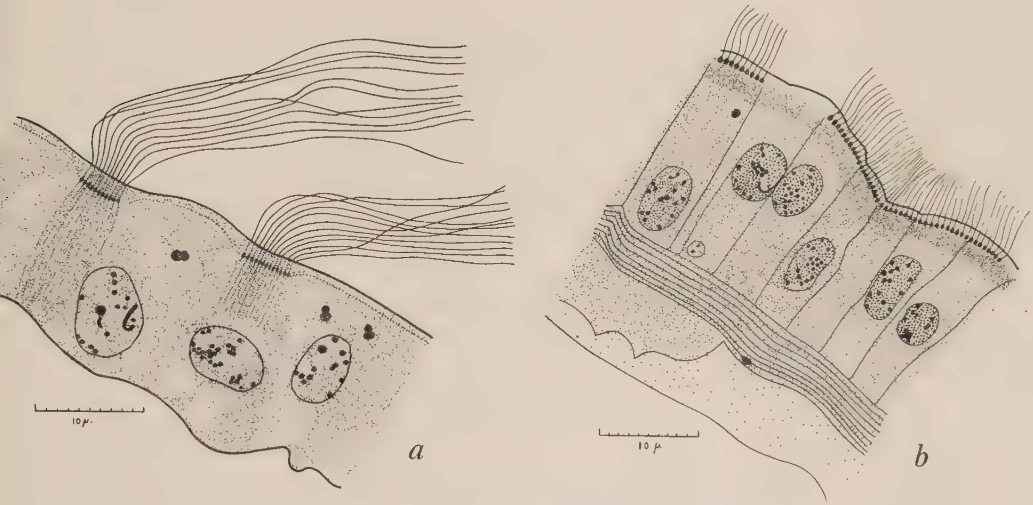


Fig. 5 *Teredo norvegica*: *a*, section through the epithelium of the visceral mass. *b*, section through the ciliated mantle tract.

There is often a slight but distinct delay before particles laid upon the visceral mass are set in motion by this scattered series of cilia, and it is possible that these cilia are under some distant control. There is a close resemblance between the spindle here described, passing from the plate of basal granules, and a spindle figured by Carter (1926), who established that certain cilia in the veliger larvae of various Nudibranchia are under nervous control.

The epithelium forming the ciliated mantle tracts differs markedly from that covering the general surface of the mantle (Fig. 5*b*). The cells are deep and columnar with distinct lateral walls. The nuclei are similar to those found in the syncytium covering the general surface of the mantle. The cytoplasm is a little more densely aggregated close to the surface, which is evenly covered with short cilia arising from a regular layer of basal granules. Very occasionally a cell is encountered which bears no cilia. There is no fibrous spindle arising from the basal granules in these cells. Many mucous glands occur in the ciliated mantle tracts.

Similar mantle tracts have been described in *Pinna* (species not determined) by Stenta (1902), in *Pinctada vulgaris* by Herdman (1904), in *Cardium*, *Mytilus*, *Ostrea* and *Pecten* by Orton (1912), and in *Arca tetragona* and *Glycymeris glycymeris* by Atkins (1936).

In *Teredo norvegica*, as in *T. megotara* and in *T. navalis* (see Lazier, 1924), the mantle is of the same thickness throughout, but in *Bankia gouldi* (see Sigerfoos, 1908) the mantle is greatly thickened median to the ciliated mantle tracts, as shown in Fig. 3 *d*, *e*, CMG, forming well-defined "ciliated mantle grooves". Sigerfoos considered that in *B. gouldi* the mantle grooves are closely opposed to the free edges of the ctenidia and that, under the combined effect of the cilia in the marginal food grooves and in the mantle groove, food particles collected from the water are driven forwards to the mouth. But it is improbable that the cilia of the mantle groove in *B. gouldi* beat forward.

Teredo megotara Hanley

The ctenidia.

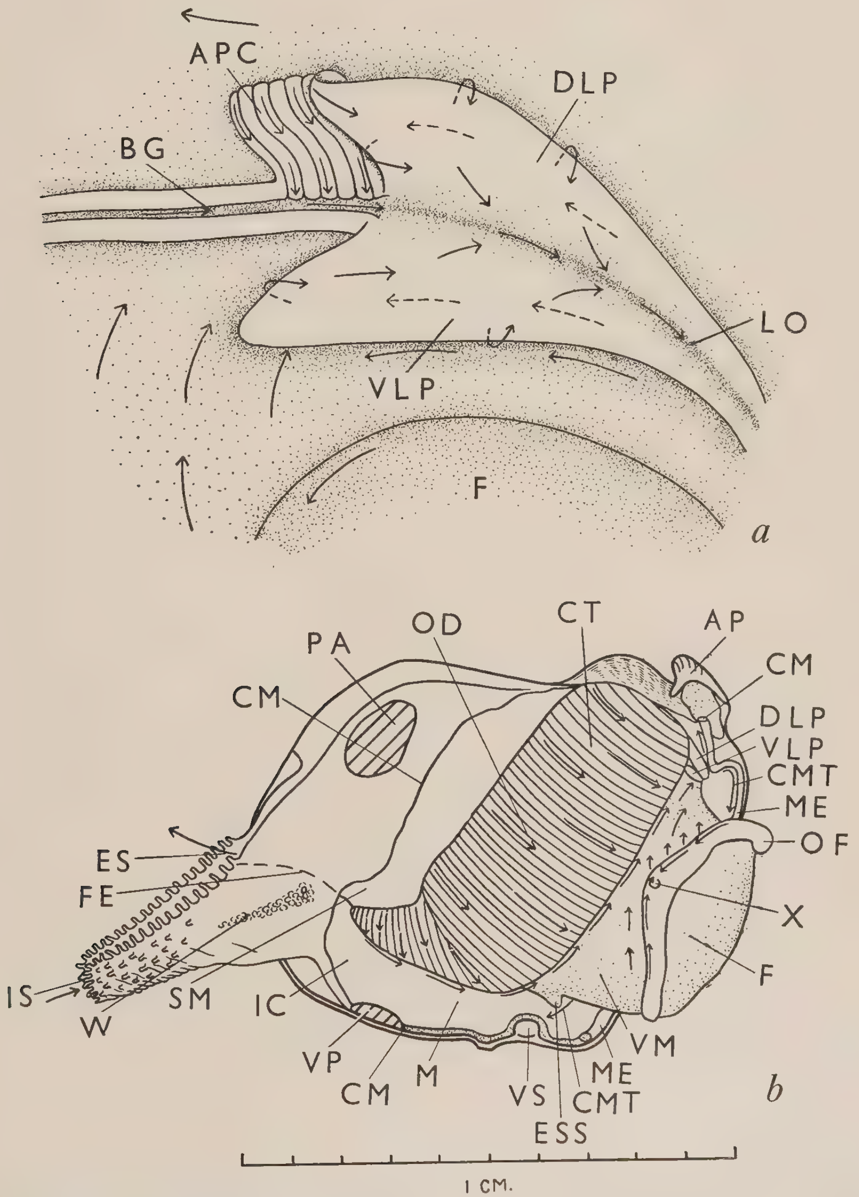
The anterior portion of the ctenidium is composed of seven ascending filaments, as shown in Fig. 6*a*, APC. The posterior portion of the ctenidium resembles that of *Teredo norvegica* in all respects save its appearance in transverse section. The ctenidial axis occupies the normal position and the ctenidium is V-shaped when seen in transverse section (Fig. 3 *f*). The ciliary currents on the ctenidium are similar to those of *T. norvegica.*

The labial palps.

In *T. megotara*, which closely resembles *T. navalis* (see Lazier, 1924), reduction of the labial palps has been carried further than in *T. norvegica*. Both the upper and lower palps are triangular (Fig. 6*a*, DLP, VLP), and they differ from those of other lamellibranchs (save *T. navalis*, as described by Lazier (1924), and *Xylophaga dorsalis*, which will be described later) in that the *inner* as well as the outer surfaces are *smooth.*

The application of carmine particles and of carborundum powder revealed that no sorting takes place on the palps, all material being passed to the mouth. The ciliary currents on the outer surfaces pass material backwards and downwards and over the free edges of the palps on to the inner surface. On the opposed surfaces the currents are directed forwards and upwards to the lateral oral groove. In Fig. 6*a* the direction of the ciliary currents on the outer surfaces is indicated by broken arrows. Material passed forwards by the branchial groove enters the lateral oral groove at the base of the palps and is rapidly borne to the mouth. Particles which fall on the visceral mass close to the labial palps pass towards these and are incorporated in the food current.

The labial palps often suffer shock following dissection, as the removal of the shell valve jerks the apophysis slightly, and this is situated in an intucking of the mantle below the palps. On such occasions the mucous string arriving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1 CM.

Fig. 6. *a*, *Teredo megotara*: anterior portion of ctenidium and labial palps: lettering as before. *b*, *Xylophaga dorsalis*: ciliary currents in the mantle cavity: AP, accessory plate; ESS, blind end of style sac; FE, floor of the exhalant siphon; ME, mantle edge; OF, opaque disk of foot; SM, suspensory membrane of ctenidium; VP, ventral pallial muscle; W, wedge-shaped mass of mucous glands; X, vortex in the ciliary currents on flank of the foot. Other lettering as before.

from the branchial groove is not accepted, due, it is thought, to some lack of continuity in the action of the cilia. This was not a normal rejection mechanism of the palps. When carmine is laid on the posterior portion of the ctenidia it travels up the branchial grooves on both sides of the visceral mass and, at the same time that material is being rejected by the palps on the dissected side, it can be seen, by moving the foot to one side, that the palps on the other side are accepting the food stream, passing both carmine and even carborundum powder along the lateral oral groove towards the mouth.

Thus no selective activity is displayed by the labial palps in *Teredo megotara*. There remains the selection displayed by rejection mechanisms of the mantle. If large quantities of carmine are added they become entangled as they pass along the marginal food groove, or along the branchial groove, with the mucous threads on the ciliated mantle tracts, and are then drawn away from the food stream by the superior strength of the rejection mechanisms.

The visceral mass and the mantle.

The ciliary mechanisms on the visceral mass and on the mantle are similar to those described for *T. norvegica*.

Xylophaga dorsalis (Turton)

The ctenidia.

The ctenidium is a large but delicate organ placed obliquely on the side of the visceral mass, as illustrated in Fig. 6*b*, OD. The posterior ends of the two ctenidia meet in the mid-ventral line near the origin of the inhalant siphon. The ctenidium is flat and homorhabdic and possesses no marginal groove. It is greatly reduced and consists of the direct lamellae (not divided into descending and ascending lamellae) of the *outer* demibranch (Purchon, 1939). The ctenidial axis runs along the ventral margin of the lamella (Fig. 3*b*, CA). The afferent branchial vein runs in the ctenidial axis (Fig. 3*b*, AB), and the efferent branchial vein traverses the dorsal margin of the lamella (Fig. 3*b*, EB).

The major portion of the ctenidium is broad and in life is held well away from the visceral mass by the large quantity of water that is held in the suprabranchial cavity. At the posterior end the filaments are much shorter than they are in the middle of the ctenidium (Fig. 6*b*). The ciliary currents are all directed downwards towards the ctenidial axis and forwards along it to the labial palps (Fig. 6*b*). The ctenidia possess no sorting mechanisms.

The labial palps.

The upper labial palp is a small spindle-shaped organ, the outer surface of which is fused to the visceral mass throughout its length (Fig. 7*a*, DLP). It is fleshy and tapers considerably distally. The lower palp is still smaller and is flat, and the outer surface is free from the visceral mass to a slight extent along its lower border. It forms a broad platform extending from the ctenidium to the mouth and is partially overhung by the upper palp (Fig. 7*a*, VLP).

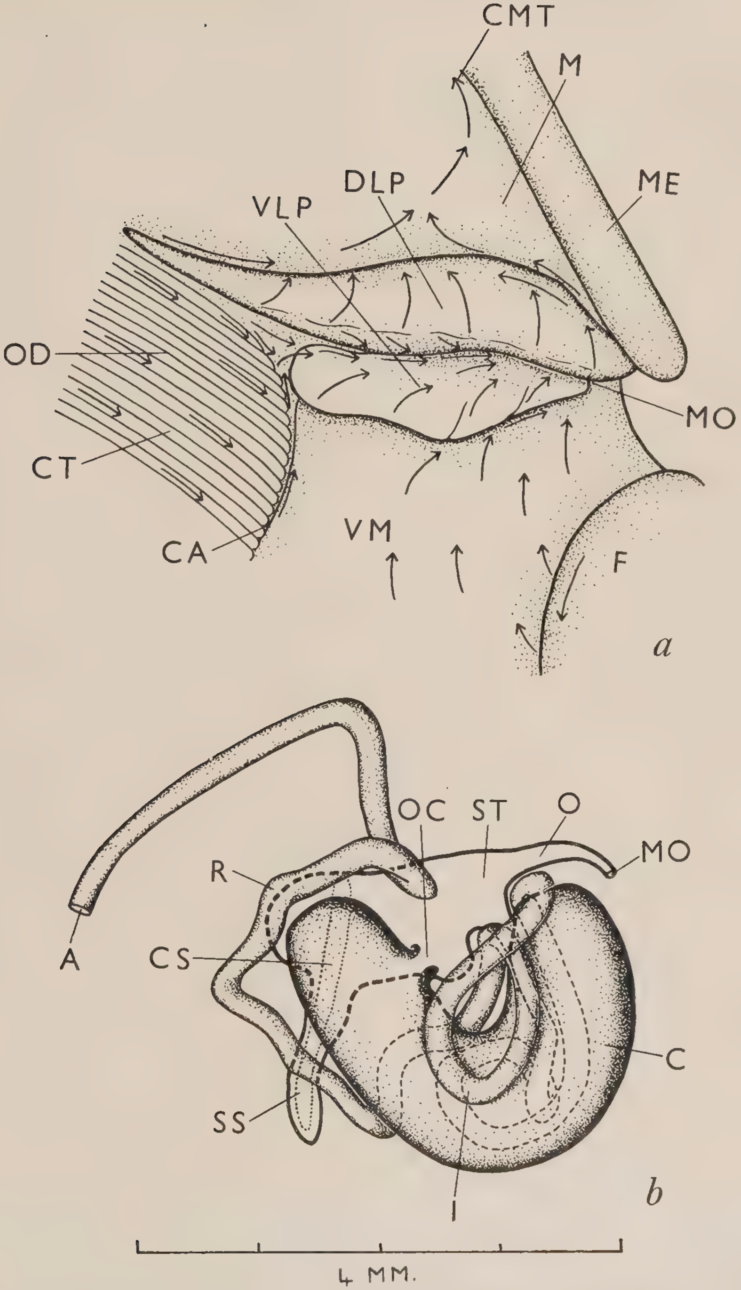
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Fig. 7. *Xylophaga dorsalis*: *a*, labial palps and adjacent organs of the right side, upper palp turned slightly upwards to expose ciliary currents on the inner surface: MO, mouth. *b*, alimentary canal reconstructed from serial sections: A, anus; C, caecum; CS, crystalline style; I, intestine; O, oesophagus; OC, opening of caecum into stomach; SS, style sac; ST, stomach; R, rectum. Other lettering as before.

The sorting mechanism on the palps is greatly reduced. On the lower palp the currents, as shown in Fig. 7*a*, carry particles upwards and forwards to the mouth (MO). Along the innermost strip of the exposed, free surface of the upper palp the cilia also beat in the direction of the mouth; but over the greater extent of its surface the cilia beat outwards, away from the oral groove and on to the mantle, where rejected material is removed by the ciliated mantle tract which arises close to the upper palp (Fig. 7*a*, CMT).

Small quantities of carmine are accepted by the palps and passed to the mouth, but slightly larger quantities come under the influence of the outward-beating cilia on the upper palp. All particles are then drawn out of the lateral oral groove and transferred to the ciliated mantle tract. In Fig. 7*a* the upper palp has been turned slightly upwards in order to expose the ciliary currents on its inner surface. The labial palps in *Xylophaga* retain to a certain degree the power of quantitative selection.

The visceral mass and the mantle.

On the sides of the visceral mass the cilia beat upwards towards the labial palps as in *Teredo norvegica* and *T. megotara* (Figs. 6*b*, 7*a*). On the ventral region of the visceral mass no ciliation was observed. On the latero-dorsal flank of the foot the cilia beat upwards. At X the cilia impart a clockwise spin to material, which quickly collects there. This mass is removed from the foot by the ciliated mantle tract.

The major portion of the mantle is unciliated, particles being efficiently removed, under normal conditions, by the ctenidia. A ciliated mantle tract arises close to the upper labial palp as already described (Fig. 7*a*, CMT) and passes downwards close to the mantle edge until it is opposite the point at which particles collect on the sides of the foot (Fig. 6*b*, X). Here the mantle tract leaves the mantle edge and passes diagonally downwards to the mid-ventral line where it meets the mantle tract of the opposite side (Fig. 6*b*, CMT). A common mantle tract passes backwards from this point to the base of the inhalant siphon (as in *Teredo* and *Bankia*, the mantle lobes are completely fused in the mid-ventral line; Fig. 6*b*, CM). The ventral region of the mantle anterior to the point where the two mantle tracts unite is weakly ciliated, and particles falling here pass slowly backwards into the ciliated mantle tracts.

THE MORPHOLOGY OF THE ALIMENTARY CANAL AND DIGESTION OF WOOD BY *XYLOPHAGA*

The anatomy of *Bankia gouldi* has been described by Sigerfoos (1908) and the morphology of the alimentary canal of *Teredo navalis* by Lazier (1924). The only contribution to our knowledge of *Xylophaga* was made by Potts (1923). Working upon an unidentified species of *Teredo*, he discovered that the digestive diverticula were of two types, one of which was specialized for the ingestion and intracellular digestion of wood fragments. He was unable to find any specialized area of the digestive diverticula in *Xylophaga*, and concluded that the development of this specialized phagocytic region of the digestive diverticula in *Teredo* was associated with "the great development of the capacity for digesting wood (which was already possessed by *Xylophaga* to some extent) and a very rapid rate of growth". He concluded that *Xylophaga*, which possesses a caecal diverticulum of the stomach, has a limited capacity for digesting wood. No evidence was given in support of these views. Harington (1921) demonstrated the presence of a cellulase in the digestive diverticula of *Teredo norvegica*. Sawdust was digested, producing glucose, but not pure cellulose in the form of filter paper. The digestion of wood by *T. navalis* was demonstrated by a different technique by Dore & Miller (1923) and Boynton & Miller (1927). Chemical analyses were made of the faecal pellets of *T. navalis* and also of the wood through which the same specimens were boring. It was shown that wood loses up to 80% of its cellulose and 15–56% of its hemicellulose during its passage through the gut of the animal. Potts (1923) reared specimens of *Teredo* (species undetermined) from an early age to maturity in water freed from plankton, and concluded that the animal is more or less independent of plankton. The work of Roch (1932) supported his conclusions.

In the Lamellibranchia food normally passes through the alimentary canal in a slow steady stream so far as external conditions permit. The rhythmical production of large quantities of wood fragments in the Teredinidae and in *Xylophaga* by boring operations therefore involves corresponding modifications in the mode of digestion and the provision of a mechanical contrivance whereby the spasmodic entrance of material into the alimentary canal is converted into a slow steady stream. The functions of the caecum—a diverticulum of the stomach only possessed by wood-boring molluscs—are possibly to provide a reserve of food upon which the animal may live after it has exhausted the supply of available timber or ceased to bore, and, more probably, to release these fragments into the stomach in a slow and continuous stream.

In the rock-boring molluscs of the family Pholadidae, the products of their boring activities are not passed through the alimentary canal. The morphology of the alimentary canal of a small specimen of *Barnea parva* (Pennant) was examined so that a comparison could be drawn between the alimentary canal of *Xylophaga* and that of a typical member of the Pholadidae. The alimentary canal of *Xylophaga* was also compared with that of *Teredo navalis* as described by Lazier (1924).

In *Xylophaga* the mouth is an oval aperture situated between the labial palps just below the anterior adductor muscle (Figs. 7 *a*, *b*, MO). The oesophagus is wide but greatly flattened dorso-ventrally; it is considerably shorter than that of *Barnea parva* (Fig. 8, O). Its inner surface is smooth anteriorly but strongly ridged posteriorly; it is lined by a ciliated columnar epithelium. The oesophagus passes through the digestive diverticula and opens into the anterior end of the stomach.

The stomach (Figs. 7 *b*, 9 *a*, *b*, ST), which is situated to the left of the median line, has an irregularly oval cavity, the surface of which is lined for the most part by the gastric shield (Fig. 9*a*, GS). The stomach of *Xylophaga* is relatively larger than that of *Barnea*. The stomach of *Teredo navalis* also is enlarged (Lazier, 1924). The stomach is lined by a ciliated columnar epithelium; the cilia are of various lengths, the longest ones being situated ventral to the gastric shield close to the point at which the crystalline style bears upon it.

The digestive diverticula consist of a number of small tubules which surround the oesophagus and lie close to the surface of the visceral mass at its anterior end. Two broad ducts open into the anterior end of the stomach (Fig. 9 *a–c*, DD). These ducts extend upwards under the umbonal beaks of the shell valves, surrounding the intucking of the mantle within which the shell ligament is situated (Fig. 9 *a*, *b*, IM, L). Cilia have not been observed in sections either in the digestive diverticula (although seen in living tissue by Potts (1923) and Yonge (1926*a*)) or in their ducts, nor have phagocytes been observed in these regions. The cells forming the digestive diverticula are small and cuboid; they normally contain large numbers of very small spherical granules of a pale yellow colour. No evidence has been produced regarding the ingestion of particles, although this may be assumed in the light of previous data (Yonge, 1926*a*). Wood fragments have never been recorded either in the digestive diverticula or in their ducts, thus confirming the statement of Potts (1923) that in *Xylophaga* the digestive diverticula are not specialized for the ingestion of wood particles.

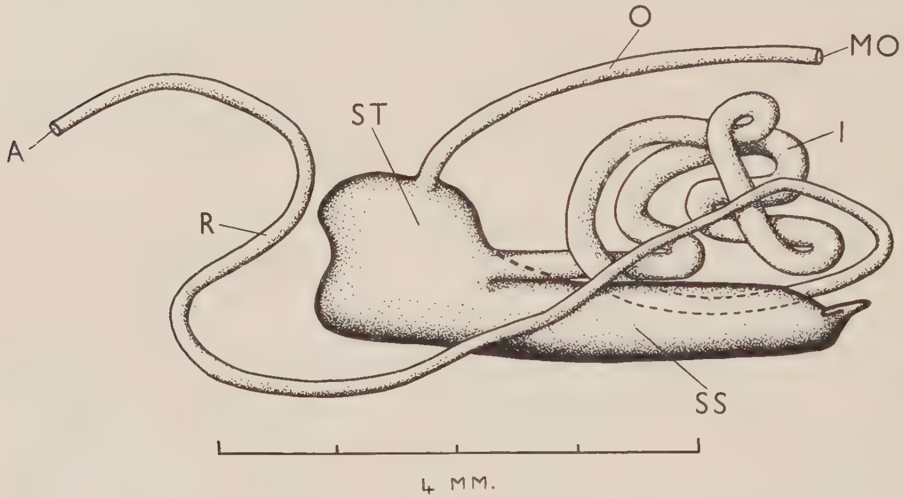

Fig. 8. *Barnea parva*: alimentary canal, reconstructed from serial sections. Lettering as before.

At the posterior end of the stomach the style sac enters on the right side (Fig. 7*b*, SS). This is small, and the crystalline style reduced (Figs. 7 *b*, 9 *a*, *c*, CS) when compared with that of *Barnea parva* (Fig. 8). Nelson (1918), who

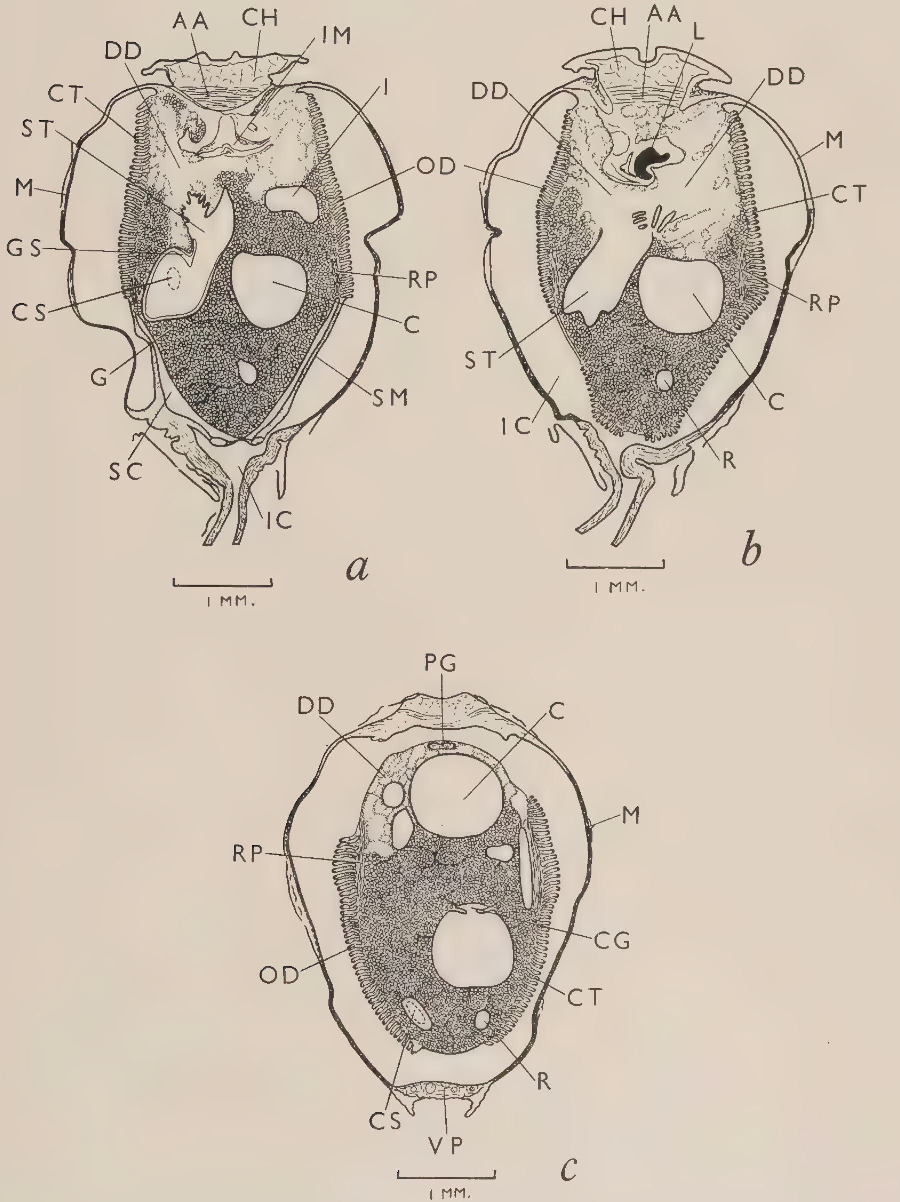
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Fig. 9 *Xylophaga dorsalis*: horizontal sections through the whole animal, *a* cut slightly above *b*, and *b* above *c*: CG, ciliated groove of caecum; DD, digestive diverticula; G, gonad tubules filled with ova; GS, gastric shield; IM, intucking of mantle; L, ligament; PG, pedal ganglion. Other lettering as before.

did not personally examine *Teredo*, referred to the style, which he described as being much smaller than that of *Pholas* and of *Martesia*.

In *Xylophaga*, as in all members of the Adesmacea, e.g. *Teredo* and *Barnea*, the style sac is completely separated from the intestine. The blind end of the sac of *Xylophaga* closely approaches the surface of the visceral mass in the mid-ventral line and forms there a slight but distinct projection into the infrabranchial cavity (Fig. 6*b*, ESS). The sac is lined by the typical ciliated epithelium. The head of the crystalline style projects across the stomach diagonally (Figs. 7*b*, 9*a*, CS) and bears upon a concavity in the gastric shield on the left side of the upper border of the stomach (Fig. 9*a*, CS).

The caecum also opens into the stomach on its right side anterior to, and at the same level as, the style sac. The caecum is a U-shaped cylinder, the opening into the stomach being on the posterior limb and not quite terminal (Fig. 7*b*, OC). The distal limb of the caecum passes close under the adhesive surface of the foot and closely approaches the oesophagus (Fig. 7*b*). Sigerfoos (1908) states that in a young specimen of *Bankia gouldi* the caecum is similarly disposed, but in an adult specimen, as in *Teredo navalis* (see Lazier, 1924), the caecum is directed backwards. The inner surface of the caecum bears two conspicuous ridges seen in transverse section in Fig. 9*c*, CG. These enclose a ciliated channel which extends downwards from the opening of the caecum along its inner wall, round the bend and for a short distance up the ascending limb. Although not determined, it is probable that the cilia in this channel beat towards the stomach and that by this means a slow continuous stream of wood fragments is conveyed from the distal limb of the caecum into the stomach.

On the opposite side of the caecum to the ciliated channel the epithelium is columnar and the surface may bear cilia or may be distended into little colourless bubbles. In this region phagocytes may often be found squeezed between the epithelial cells, or at the base of the epithelium. The remainder of the epithelium lining the caecum is shallow and bears no cilia. Phagocytes have never been seen in this region. In serial sections the caecum is usually distended with wood fragments amongst which phagocytes occasionally occur. In some cases, however, phagocytes are abundant in the lumen, which is then almost entirely filled with bacteria. Phagocytes also occur occasionally in the stomach, though never in the digestive diverticula or in their ducts. Bacteria form a large percentage by volume of faeces in the rectum. When the caecum is heavily laden with wood shavings it is difficult to determine whether bacteria are present, but in some serial sections it appears as if bacteria are present as well as wood fragments.

Lazier (1924) showed that in *T. navalis* the caecum is apparently not ciliated but possesses a conspicuous two-coiled typhlosole capable of writhing movements. He thought that wood fragments were moved in and out of the caecum by muscular activity of the typhlosole and of the caecum walls. No such typhlosole occurs in *Xylophaga*.

The orifice between the stomach and the caecum in *Teredo navalis* resembles that of *Xylophaga* in possessing two lateral infoldings of the wall, this arrangement presumably providing for simultaneous ingress and egress of wood fragments. The right fold in *Teredo navalis* is continuous with the caecal typhlosole. In *Xylophaga* the two folds are continuous with the ridges which lie on the inner wall of the caecum.

The opening from the stomach into the intestine in *Xylophaga* is situated at the anterior end of the stomach on the left side of the ventral wall. The course of the intestine is complicated and can best be understood by reference to Fig. 7*b*. The intestine coils once on the left side of the stomach, rises and passes over the distal extremity of the caecum, coils once on the right side of the caecum and returns to the left side below the stomach. It then passes behind the style sac and merges into the rectum which passes forwards and upwards to the pericardium. The rectum travels upwards through the pericardium and, unlike that of *Teredo*, penetrates the ventricle.

Typically, lamellibranchs possess a typhlosole which extends throughout the intestine. Such a typhlosole is found in *Barnea parva*. Sigerfoos (1908) found that in *Bankia gouldi* the typhlosole extends throughout the intestine. According to Lazier (1924) *Teredo navalis* possesses a typhlosole only in the anterior portion of the intestine, which is greatly dilated in this region. A typhlosole has also been noted in certain portions of the intestine of *T. norvegica*, though the whole of the intestine has not been examined. In *Xylophaga dorsalis* no intestinal typhlosole is present; for this reason it is difficult to determine at which point the intestine passes into the rectum. In spite of the absence of an intestinal typhlosole, the faeces are well consolidated. They are extruded into the supra-branchial cavity and accumulate, as already described, in the posterior end of the burrow.

The absence in *Xylophaga* of a portion of the digestive diverticula specialized for the intra-cellular digestion of wood fragments (Potts, 1923) has led to a general belief that this species is incapable of digesting wood (Yonge, 1937, 1938).

Evidence is here produced which suggests that *Xylophaga* may be able to derive some nutriment from the wood through which it bores. It must be emphasized, however, that *Xylophaga* under certain circumstances is certainly independent of wood as a source of food. Specimens of *Xylophaga* living in the gutta-percha sheaths of submarine telegraph cables must subsist entirely upon plankton or other matter suspended in the water. Such specimens must penetrate metal wrappings before entering the gutta percha, unless they attack the cables only at points where the metal casings have been damaged. An outer wrapping of brass tape is now used to prevent such damage.

In order to determine whether *Xylophaga* possesses a cellulase, approximately twenty-four individuals of various sizes were extracted in sea water after grinding up with clean silver sand. The extract was diluted to 30 c.c. with clean sea water and divided into three equal parts. The substrate consisted of fine sawdust previously treated three times with boiling water to remove any soluble sugars. The experiment and two controls were incubated at 30° C. for 14 days and the glucose present then estimated by the technique used by Boyland (1928). Full details are given in Table I.

TABLE I

Experiment	10 c.c. extract + 0·2 g. sawdust	4·725 g. glucose
Control 1	Ditto boiled	3·213 g. glucose
Control 2	Ditto without substrate	3·517 g. glucose

The glucose present in control 1 represents that originally present in the extract, that in control 2 represents this amount together with any produced by autolysis during the period of the experiment. The somewhat greater quantity of glucose present in the experiment indicates, although not with any certainty, the possibility of a cellulase. Further experiments with greater numbers of animals are necessary before this point can be settled.

If *Xylophaga* is capable of digesting wood the question arises as to where digestion takes place. In *Teredo* it was shown by Potts (1923) that wood is ingested in regions of the digestive diverticula specialized for this purpose. He also showed that these are absent in *Xylophaga*, a fact which the present investigation has confirmed. It is not impossible that the bacteria found in the caecum may feed on the wood and then be ingested by the phagocytes, which, as originally shown by Yonge (1926*a*, 1926*c*), play so great a part in the digestive processes of the Lamellibranchia. Further work is indicated on the nature of these bacteria and their presence in other wood-boring species. Final decision on the capacity of *Xylophaga* to digest wood will have to await the results of such work.

SEX CHANGE AND SEXUAL DIMORPHISM IN *XYLOPHAGA*

It is known that hermaphroditism occurs in *Bankia gouldi* (see Sigerfoos, 1908), *Teredo norvegica* (see Yonge, 1926*b*) and *T. navalis* (see Coe, 1933, 1934, 1935, 1936). To determine its possible occurrence in *Xylophaga dorsalis*, smears were made of the gonads of ninety-six specimens, while large numbers of specimens of many different sizes were sectioned to show the condition of the gonads. The greatest antero-posterior dimension of the shell of each individual examined was recorded, and the results obtained are given in Table II.

TABLE II. THE RELATION BETWEEN SIZE AND SEX IN *XYLOPHAGA DORSALIS*

Antero-posterior length of shell in mm.	Number of males	Number of hermaphrodites	Number of females
0–3	16	1	1
3–4	9	1	7
4–5	15	—	13
5–6	7	—	6
6–7	3	—	3
7–8	4	—	6
8–9	—	—	3
9–10	—	—	1

It will be seen that, of eighteen specimens not exceeding 3 mm. in length, all were males with the exception of one hermaphrodite and one female. A second hermaphrodite specimen was present in the next size group, while the remaining animals between 3 and 8 mm. long were fairly evenly divided between males and females. The four specimens which exceeded 8 mm. in length were all females.

X. dorsalis appears, therefore, to be a protandric hermaphrodite. The young specimens are male, change to the female sex occurring when they reach a length of about 3 mm.; but this change may apparently be delayed, and therefore animals of medium length are about evenly divided between the sexes.

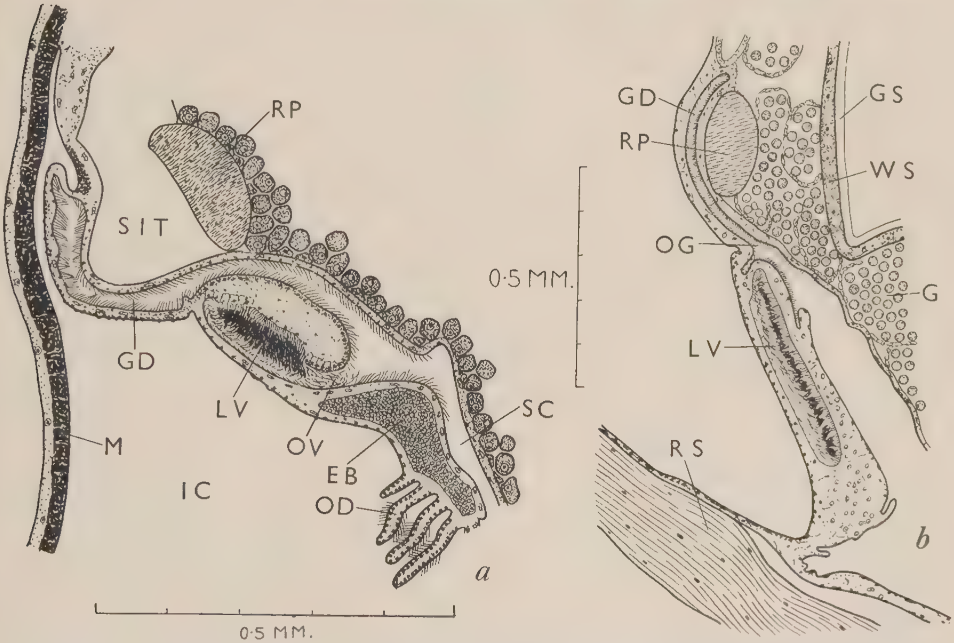

Fig. 10. *Xylophaga dorsalis*: *a*, transverse section through the vesicula seminalis: GD, genital duct; LV, lumen of vesicula seminalis; SIT, split in tissues. *b*, longitudinal section through the vesicula seminalis: OG, opening of genital duct; WS, wall of stomach. Other lettering as before.

It is possible that individuals may undergo more than one change in sex, so that the largest size groups may consist of individuals which are female for the second time. Coe (1933–6) believes that such alternation of sexes occurs in *Teredo navalis*.

Examination of serial sections of various specimens confirmed these observations. In Fig. 12*a* is shown the appearance of the gonad in a hermaphrodite individual 3 mm. long. The lumina of the gonadial tubules are filled with spermatocytes and ripe spermatozoa, whilst the walls of the tubules are lined with developing oogonia of various sizes. It is less easy to demonstrate a change of sex from female to male because this is thought to occur relatively quickly (Coe, 1933). Evidence has been obtained, however, from serial sections of an individual 5 mm. long, of a change of the gonad from female to male. In this animal the tubules were filled with spermatocytes and spermatozoa, with no signs of oogonia. The animal, therefore, was in the male phase. In its genital ducts, however, a small number of ripe ova was found, and since there was no sign of change of sex in the gonads, it was concluded that these ova were formed in a female phase prior to the existing male phase. This may be regarded as presumptive proof that a second change of sex, from female to male, may occur. Since the largest specimens examined were all females, it is possible that a further change of sex may occur.

In *Xylophaga* the genital duct is short, passing round the retractor muscle of the foot (Fig. 10 *a*, *b*, RP) and opening at the base of the suspensory membrane of the gill into the supra-branchial cavity anterior to the opening of the kidney duct (Fig. 10*a*, GD; Fig. 10*b*, GD, OG). It is lined by a ciliated epithelium which extends for a short distance over the suspensory membrane of the gill. Situated in the suspensory membrane close to the genital opening is a cylindrical cavity with an anterior opening. The cavity is lined by a ciliated epithelium which is continuous with that extending from the genital duct over the suspensory membrane. The opening of this ciliated pocket is directed ventrally (Fig. 10*a*, OV) and is situated close to the genital opening. The lumen of this organ (Fig. 10*a*, *b*, LV) is filled with spermatozoa, which are tightly packed and orientated with their heads pointing towards the ciliated epithelium lining the cavity. Irregularly disposed spermatozoa can be seen at the orifice of the organ (Fig. 10*a*, OV), and these may have been entering or leaving the organ.

It is considered that the organ is a vesicula seminalis, and that it is loaded with spermatozoa when these are discharged at the end of the male phase of the gonad. No other records of such an organ in the Lamellibranchia have been found, and it is possible that *Xylophaga* is peculiar in the possession of a pair of vesiculae seminales. *X. dorsalis* presumably shares this distinction with other members of the genus, e.g. *X. praestans*, *X. indica*, and *X. globosa*.

The vesiculae seminales are present in specimens of all sizes, even in the smallest ones examined, in both sexes, and always contain a considerable quantity of spermatozoa. The method by which they are filled with spermatozoa will be discussed later. Whether self-fertilization occurs in *Xylophaga* to the exclusion of cross-fertilization is difficult to determine, but it appears certain that the possession of these organs renders self-fertilization possible.

Xylophaga is peculiar among the Lamellibranchia not only in the possession of vesiculae seminales, but also for the development of an unusual glandular organ in the supra-branchial cavity. This "accessory genital organ", as it will be here called, is found in a fully developed condition in the male phase only, thus making *Xylophaga* one of the few recorded cases of external sexual dimorphism in the Lamellibranchia. It lies suspended from the posterior surface of the posterior adductor muscle (Fig. 11) and when fully developed consists of a large fleshy peduncle (Fig. 11*c*, PE) which expands distally into a broad, slightly bilobed blade or lamella (Fig. 11*c*, LA). The dorsal border of the peduncle encircles the rectum close to the anus. The blade is pressed closely against the posterior and lateral surfaces of the visceral mass. Fig. 11*c* shows the organ removed from the visceral mass, and in it the two lobes of the blade are pressed close to one another. In serial sections, the fully developed organ more or less completely occludes the supra-branchial cavity.

The posterior surface of the blade, and that of the peduncle, are sparsely ciliated, the weak ciliary currents being directed upwards towards the posterior adductor muscle. The anterior surface of the blade, which is closely pressed against the visceral mass, is composed of a very shallow unciliated epithelium (Fig. 12 *b*, *c*). The cilia on the posterior surface of the blade, are seldom visible in sections.

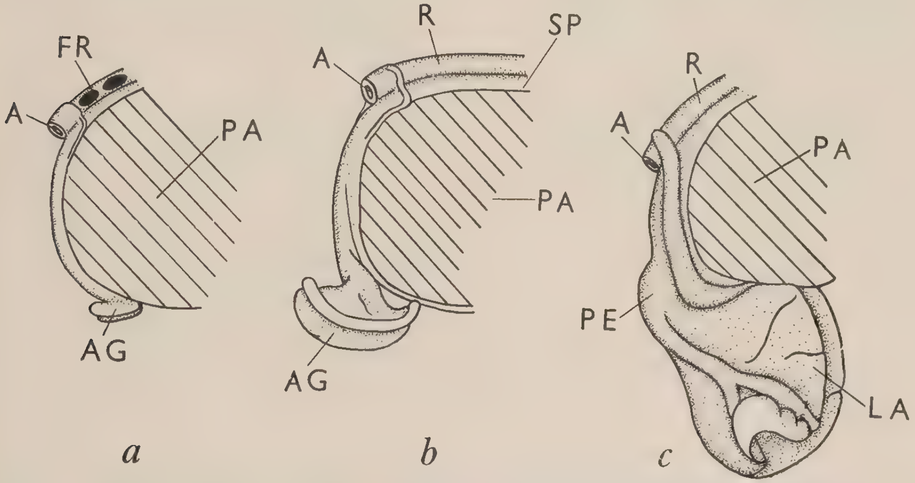

Fig. 11. *Xylophaga dorsalis*: the accessory genital organ in various stages of development, seen from the right side: *a*, small, from a female; *b*, medium, from a female, or hermaphrodite; *c*, large, from a male: AG, accessory genital organ; FR, faeces in rectum; LA, lamella, or blade, of accessory genital organ; PE, peduncle of accessory genital organ; SP, dorsal surface of posterior adductor. Other lettering as before.

The organ is of very variable size, being fully developed only in male specimens; occasionally it is of an intermediate size in female specimens—suggesting a recent change of sex—but it is always medium in size in hermaphrodite specimens. Data on this matter are summarized in Table III, which indicates the intimate relationship between the size of this accessory genital organ and the sex of the individual.

Serial sections through the accessory genital organ show that it is glandular, the posterior surface being composed of mucoid cells. This layer occupies about a third of the thickness of the blade (Fig. 12*b*). No glands open on to the anterior surface. The remainder of the lamella is composed of a glandular

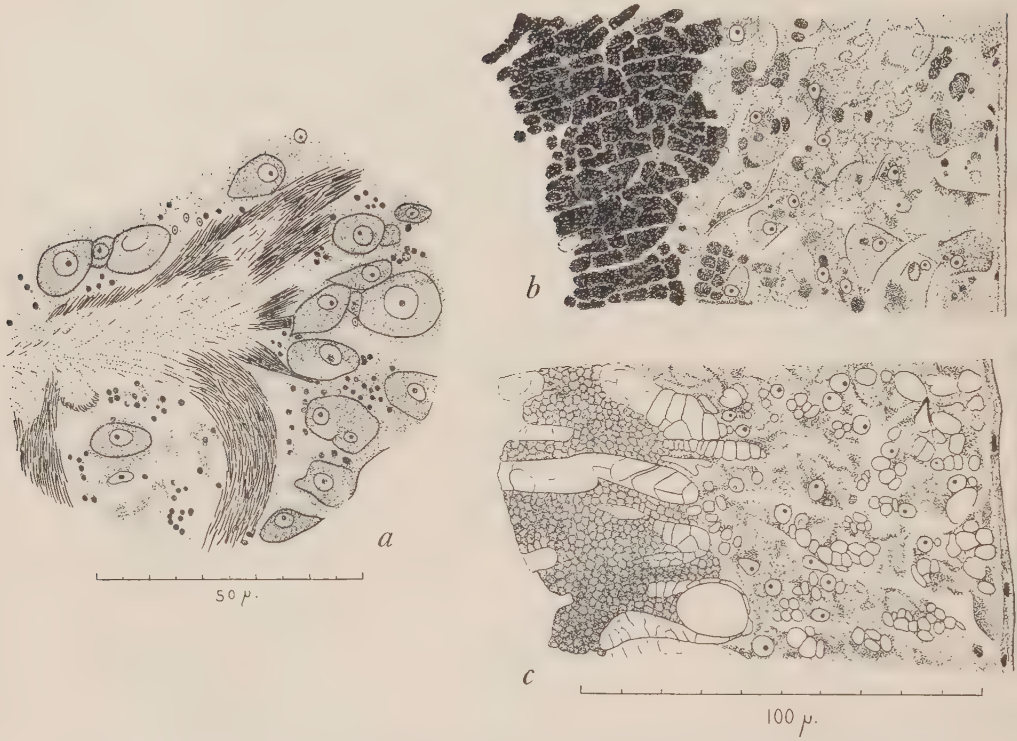

Fig. 12. *Xylophaga dorsalis*: transverse sections: *a*, through the gonad during the initial change from the male to the female phase; *b*, through the blade of the accessory genital organ, seen here in a ripe condition; *c*, through the blade of the accessory genital organ, in a spent condition.

TABLE III. THE RELATION BETWEEN THE SIZE OF THE ACCESSORY GENITAL ORGAN AND THE SEX OF THE ANIMAL

Antero-posterior length of shell in mm.	Size of accessory genital organ			
	Large	Medium	Small	Absent
0–3	16 ♂	1 ☿	—	1 ♀
3–4	7 ♂	2 ♂, 1 ☿, 3 ♀	1 ♀	3 ♀
4–5	13 ♂	2 ♂, 1 ♀	3 ♀	9 ♀
5–6	7 ♂	1 ♀	1 ♀	4 ♀
6–7	3 ♂	—	2 ♀	1 ♀
7–8	4 ♂	—	3 ♀	3 ♀
8–9	—	—	2 ♀	1 ♀
9–10	—	—	1 ♀	—
Total	50 ♂	4 ♂, 2 ☿, 5 ♀	13 ♀	22 ♀

tissue which is for the most part stained but lightly with Delafield's haematoxylin. The cells are roughly fusiform in shape, and are shown in transverse section in Fig. 12 *b*, *c*. Their nuclei are easily distinguished, for they have a distinct nuclear membrane and a large central nucleolus. The secretion arises close to the nucleus, and when first formed consists of a lightly stained packet which later acquires darkly stained walls surrounding unstained contents. The number of these packets of secretion increases until finally a reticulate mass of bubbly appearance is formed. The secretion must pass to the posterior surface of the blade, which is deeply folded close to the peduncle. In these folds strands of mucus are frequently seen. Comparison of a number of series of sections shows that the organ secretes as a whole. Either the posterior surface is loaded with secretion or all the secretion has been discharged, leaving the cells at the posterior surface empty (Fig. 12*c*). When the organ has secreted it presumably commences to degenerate.

It is probable that when spermatozoa are discharged from the gonad duct, owing to the pressure of the ripe gonads, they are forced past the openings of the vesiculae seminales, and fall upon the posterior surface of the supra-branchial organ. It is at this stage that the accumulation of mucus is discharged, and in it the spermatozoa become entangled. By the action of the cilia upon the posterior surface of the blade and peduncle the spermatozoa are driven upwards and conveyed to the openings of the vesiculae seminales. The spermatozoa are then passed into the lumina of these organs by the cilia on the suspensory membranes. Evidence has been found which supports this theory. In one series of sections both spermatozoa and spermatocytes were found entangled in mucous threads on the posterior side of the blade. The presence here of spermatocytes indicates that the spermatozoa were those produced by the animal itself, and were not foreign spermatozoa collected from the inhalant current.

When the vesiculae seminales are fully loaded the function of this accessory genital organ has been completed, and its degeneration commences. Residual sperm in the gonads may either be discharged or resorbed.

With the degeneration of the accessary genital organ the volume of the supra-branchial cavity is greatly increased and at the same time the animal changes to the female phase. It is not known whether *Xylophaga* incubates its larvae; but this occurs in some species of *Teredo* (Hatschek, 1880; Calman, 1919), and in view of the probable value of such a habit to animals inhabiting driftwood, it is probable that the same is true of *Xylophaga*. If so there is now available space for the larvae in the supra-branchial cavity. As shown in Table III, the accessary genital organ is present in all males whatever their size; thus if the animals do change sex more than once it must be redeveloped during the second male phase.

The possession of both these organs is probably of great survival value to *Xylophaga*. The animals live in isolated communities in driftwood, and the chance of fertilization might be slight were eggs and sperms discharged freely into the sea. The retention of the sperms produced during the male phase will ensure fertilization of eggs produced during the later, female phase and so overcome this danger. Sigerfoos (1908) recorded that in male specimens of *Bankia gouldi* there is a great development of mucous glands in the roof of the supra-branchial cavity, but there is no record in any species of *Teredo* of any glandular organ. It is by no means impossible that *Bankia* and *Teredo* may also possess vesiculae seminales which have hitherto been overlooked.

The Systematic Position of *XYLOPHAGA*

Consideration of the shell features of *Xylophaga* led systematists to the conclusion that, although these superficially resembled those of the Teredinidae, the genus was more nearly related to the Pholadidae, in which it has accordingly been included (Adams, H. & A., 1853–8; Paetel, 1890; Pelseneer, 1906; Thiele, 1926). But examination of the anatomy of *Xylophaga* gives no support to this view. Whilst showing affinities both with the Teredinidae and the Pholadidae, *Xylophaga* also possesses certain specialized characters of sufficient importance to justify the creation of a new family for its inclusion.

In the following important points *Xylophaga* has undoubted affinities with the Pholadidae.

With the exception of the siphonal process, the animal is entirely covered by the shell valves (Figs. 2*a*, 6*b*), its burrow is never lined with a calcareous deposition, nor does the animal possess pallets as do the members of the Teredinidae. *Xylophaga* possesses a pair of accessory plates (Fig. 6*b*, AP), though their absence in the Teredinidae is probably due to reduction. The rectum of *Xylophaga* passes through the ventricle (Fig. 13*a*, R), which it does not in the Teredinidae. Finally, the visceral ganglia occupy their normal position in *Xylophaga*, on the ventral surface of the posterior adductor muscle. In the Teredinidae, owing to the great elongation of the visceral mass, these ganglia are displaced backwards and lie far behind the posterior adductor.

The above are outweighed, however, by the following affinities between *Xylophaga* and the Teredinidae.

Xylophaga bores normally in wood (Fig. 1*a*), never in stone; its shell in its general appearance and fragility is not unlike that of *Teredo*. The ctenidia of *Xylophaga* (Figs. 3*b*, 6*b*) and of the Teredinidae (Figs. 2*b*, *c*, 3, 4) are composed of only one demibranch (Atkins, 1937*b*; Ridewood, 1903). This is in each the outer demibranch (Purchon, 1939). In addition there are no ctenidial sorting mechanisms in *Teredo* or in *Xylophaga*.

The labial palps in the Teredinidae have undergone various degrees of reduction (Fig. 2*c*, LP; Figs. 4, 6*a*, DLP, VLP) and may possess no ciliary sorting mechanisms; those of *Xylophaga* (Figs. 6*b*, 7, DLP, VLP) are also greatly reduced and have poor powers of selection. The ctenidia of the Pholadidae possess both inner and outer demibranchs, their labial palps are highly organized and the ciliary mechanisms on the ctenidia and the palps are highly developed (Kellogg, 1915). The alimentary canal of *Xylophaga* possesses modifications similar to those in the Teredinidae for the acceptance of large quantities of wood fragments produced spasmodically. The oesophagus is short, the stomach is large and bears a conspicuous caecum within which wood shavings are stored. The style sac and the crystalline style are reduced in size (Fig. 7*b*, SS, CS)—contrast the large style sac of *Barnea* (Fig. 8, SS) and of *Martesia* (see Nelson, 1918). The intestinal typhlosole has undergone various degrees of reduction in the Teredinidae (Sigerfoos, 1908; Lazier, 1924); it is absent in *Xylophaga*, but well developed in *Barnea*, a member of the Pholadidae.

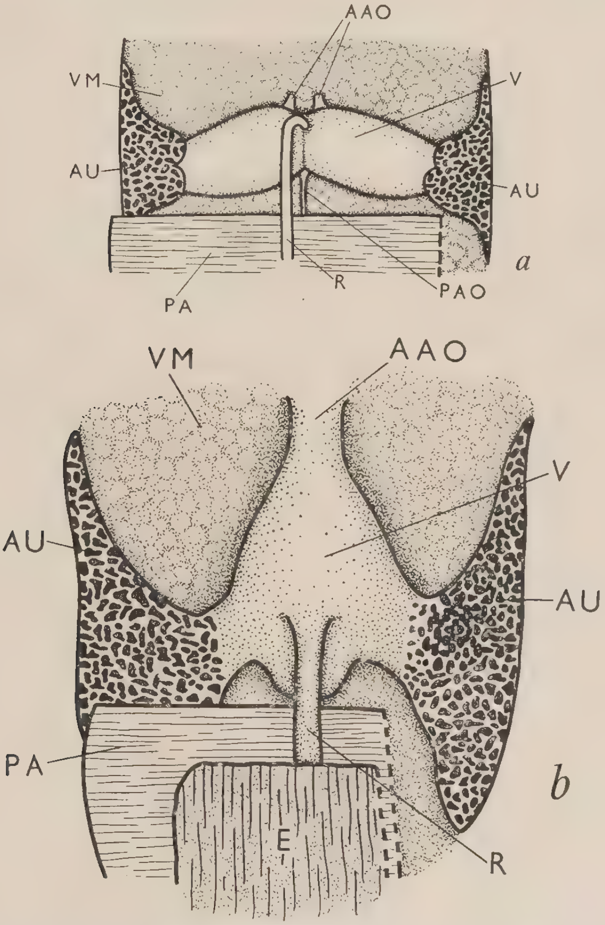

Fig. 13. Dorsal view of the organs in the pericardium. *a*, *Xylophaga dorsalis*; *b*, *Barnea parva*: AAO, anterior aorta; AU, auricle; E, epithelium of the siphonal process; PAO, posterior aorta; V, ventricle. Other lettering as before.

It has been shown that various members of the Teredinidae are protandric hermaphrodites (Sigerfoos, 1908; Yonge, 1926*b*; Coe, 1933–6), and *Xylophaga* also is a protandric hermaphrodite. This condition has never been demonstrated for any member of the Pholadidae. Various members of the Teredinidae incubate the larvae (Sigerfoos, 1908; Calman, 1919), and it is not improbable that *Xylophaga* also incubates its larvae, though this remains to be proved.

Finally, the structure of the ventricle of *Xylophaga* more nearly resembles that of *Bankia gouldi* (see Sigerfoos, 1908) than that of *Barnea parva*—in spite of the fact that it is traversed by the rectum. The ventricle of *Xylophaga* is short from front to back (Fig. 13*a*, V), it possesses two delicate anterior aortae which pass downwards into the visceral mass from a short median lobe which lies in front of the rectum (Fig. 13*a*, AAO), and a single posterior aorta (Fig. 13*a*, PAO) which passes under the posterior adductor muscle. The ventricle itself is short from back to front and is produced laterally into two conspicuous lobes which communicate with the auricles (Fig. 13*a*, AU). The ventricle of *Barnea parva* (Fig. 13*b*, V) is unlike that of *Xylophaga*; it possesses the typical fusiform shape, long in the antero-posterior plane, in which the rectum traverses the ventricle (Fig. 13*b*, R). The heart of *Bankia gouldi* (Sigerfoos, 1908) is deeply bilobed. In the young individual (Fig. 14*a*) the auricles are not attenuated, nor is the ventricle drawn out in the antero-posterior axis; it is more deeply lobed than that of *Xylophaga*. It is also morphologically upside down. Owing to the great length of the adult animal, the heart when fully developed (Fig. 14*b*) is quite unlike that of *Xylophaga*. The anterior and posterior aortae arise close to one another at the anterior end of the heart (Fig. 14*b*, AAO, PAO), and the auricles, which are greatly attenuated, communicate with two posterior lobes of the ventricle. In *Bankia gouldi* the ventricle of a young specimen, 2 mm. in length, is laterally bilobed, as it is in *Xylophaga*. This is doubtless a modification due to the globose shape of the two animals. In larger specimens of *Bankia gouldi* the antero-posterior elongation of the body necessitates a departure from the laterally expanded ventricle of the young animal.

There remain a number of characters in which *Xylophaga* differs both from the Teredinidae and from the Pholadidae. These characters show such a high degree of specialization that it is proposed to transfer *Xylophaga* from the Pholadidae and place it alone in a new family in the order Adesmacea, more closely related to the Teredinidae than to the Pholadidae.

Although it is typical of a member of the Adesmacea that the shell bears an apophysis upon which the retractor muscles of the foot are inserted, as in *Pholadidea penita* Conrad (Lloyd, 1897) (Fig. 14*c*, SA) and in *Teredo norvegica* (Fig. 14*e*, SA), *Xylophaga* possesses no shell apophysis (Fig. 14*d*), and the retractor muscles of the foot are inserted upon the shell in the primitive position, anterior to the insertion of the posterior adductor muscle. The siphonal process of *Xylophaga* is specialized, the exhalant siphon being reduced and opening within the burrow (Figs. 2*a*, 6*b*, ES). While there is some slight evidence that *Xylophaga* may resemble the Teredinidae in the possession of a cellulase, it certainly possesses no regions of the digestive diverticula exclusively specialized for wood ingestion.

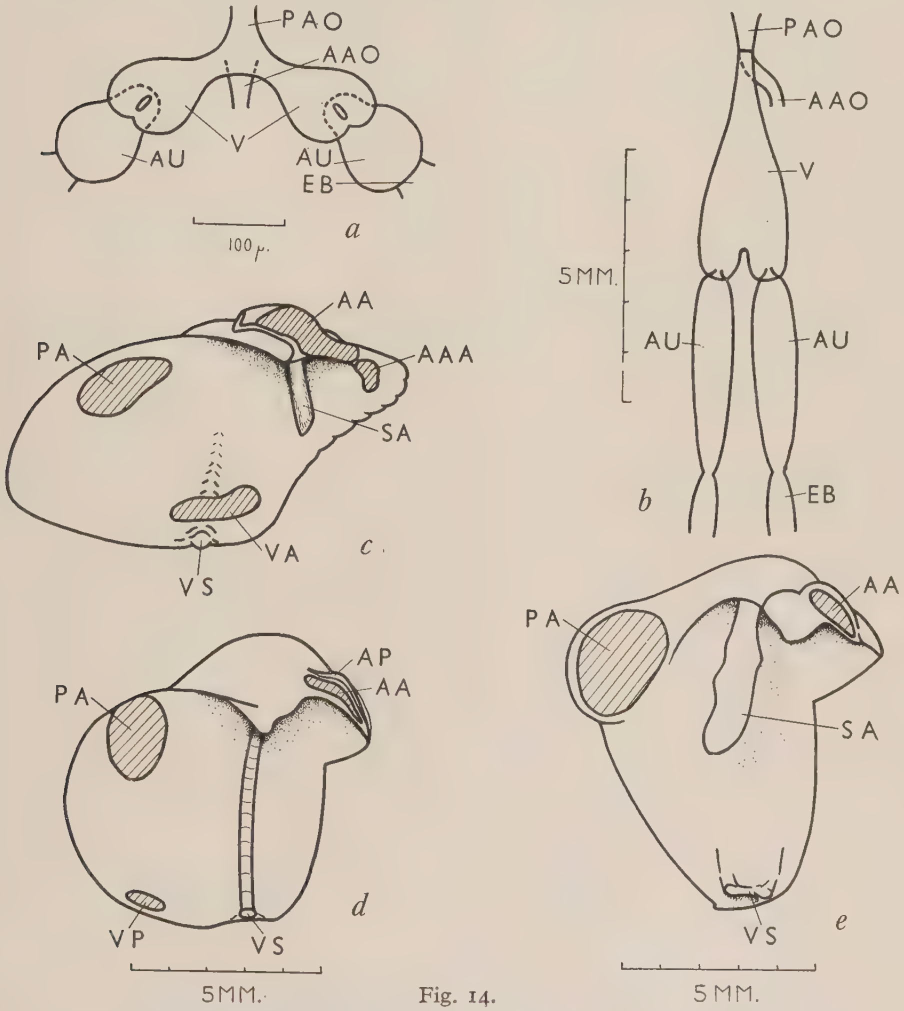
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Fig. 14.

a, *Bankia gouldi* (after Sigerfoos): dorsal view of the heart of a young individual.
b, *Bankia gouldi* (after Sigerfoos): dorsal view of the heart of an adult individual.
c, *Pholadidea penita* (after Lloyd): shell and musculature.
d, *Xylophaga dorsalis*: shell and musculature.
e, *Teredo norvegica*: shell and musculature.
AAA, accessory anterior adductor; SA, shell apophysis; VA, ventral adductor. Other lettering as before.

Apart from shell characters, the most important differences between *Xylophaga* and the Teredinidae are the modifications in the reproductive system which render self-fertilization possible, namely, the vesiculae seminales and the accessory genital organ, the presence of which appears to be correlated with the isolated life of the colonies of *Xylophaga*. Consideration of the shell and musculature of *Xylophaga* suggests possible homologies with regard to the pallets and pallet muscles of the Teredinidae. Lloyd (1897) showed that in *Pholadidea penita* (Fig. 14*c*) in addition to the anterior and posterior adductor muscles, there are auxiliary adductor muscles; of these the accessory anterior adductor (Fig. 14*c*, AAA) was probably split off the anterior adductor (Fig. 14*e*, AA). Close to the ventral articulation of the shell (Fig. 14*c*, VS) is a ventral adductor muscle (Fig. 14*c*, VA).

In *Xylophaga* no division of the anterior adductor (Fig. 14*d*, AA) has occurred. At the postero-ventral border of the shell, behind the ventral articulation of the shell is a ventral pallial muscle (Figs. 6*b*, 14*d*, VP) composed largely of longitudinal fibres, but also possessing a few transverse and dorso-ventral muscle fibres. In *Teredo norvegica* (Fig. 14*e*) the postero-ventral adductor, or ventral pallial muscle is present. In all other members of the Teredinidae the postero-ventral margin of the shell is emarginated.

It is suggested that the pallets in the Teredinidae are derived from this postero-ventral corner of the shell valves, and that the pallet muscles of the Teredinidae, the ventral pallial muscle of *Xylophaga*, and the ventral adductor of *Pholadidea* are homologous.

With regard to the accessory plates possessed by all members of the Pholadidae (not shown in Fig. 14*c*) and also by *Xylophaga* (Fig. 14*d*, AP), in the Teredinidae an anterior pallial fold, similar to that which in the Pholadidae bears the accessory plates, extends forwards over the shell valves in an antero-dorsal position (Fig. 2*c*, AF). It is therefore probable that the absence of accessory plates in the Teredinidae is secondary.

It is proposed to call the new family to which the genus *Xylophaga* is to be transferred, the Xylophaginidae. The affinities of the families in the order Adesmacea may then be summarized as follows:

	Adesmacea	
Common origin	Xylophaginidae Teredinidae	Wood-boring Lamellibranchia
	Pholadidae	Rock-boring Lamellibranchia

The affinities of *Xylophaga* are shown in tabular form in Table IV.

Discussion

If it be assumed that the absence of a shell apophysis and the insertion of the pedal retractor muscles upon the shell in *Xylophaga* are primitive characters it follows that *Xylophaga* departed from the common stock of the Adesmacea before the development of the shell apophysis, as shown below in Fig. 15.

On this theory it is evident that reduction of the ctenidium to a single demibranch in the Xylophaginidae and in the Teredinidae occurred separately, which is quite possible, and that either the caecum of the stomach was also separately evolved in these two families, or that the absence of the caecum in the Pholadidae is due to reduction. This is inherently improbable and it is therefore contended that the absence of the shell apophysis in *Xylophaga* is secondary and *not primitive*.

The implications of this second and more probable view are shown in Fig. 16. The common stock of the Adesmacea was rock-boring, and from this arose a wood-boring stock which was to evolve into the Xylophaginidae and Teredinidae. With the evolution of a wood-boring habit a stomach caecum was formed as a device converting the spasmodic production of large quantities of wood fragments into a slow steady stream passing through the alimentary canal. Due to the pelagic life in driftwood, the animals seldom, if ever, encounter the heavy sedimentation and turbidity which must be endured by rock-dwelling animals. The ctenidia and labial palps were thus reduced in structure and in function.

TABLE IV. THE AFFINITIES OF *XYLOPHAGA*

Affinities with the Teredinidae	*Specialized characters*	*Affinities with the Pholadidae*
Bores chiefly in wood, and never in stone	No shell apophysis; pedal retractor muscles inserted upon shell in primitive position	Body enclosed by shell valves
Proportions, sculpture, and fragility of shell valves	Reduction of exhalant siphon Faecal accumulation in burrow	Two accessory plates No pallets
Ctenidia possess outer demibranch only and no ctenidial sorting mechanisms	Method of wood digestion: by bacteria?	No calcareous lining to burrow Rectum passes through ventricle
Labial palps greatly reduced	—	—
Products of boring activity passed through alimentary canal	Vesiculae seminales and accessory genital organ permit self-fertilization	Visceral ganglia in normal position
Short oesophagus; conspicuous stomach; caecum to stomach; small style sac; intestinal typhlosole absent	—	—
Possible digestion of wood?	—	—
Ventricle laterally bilobed	—	—
Alternation of sex	—	—
Possible incubation of larvae	—	—

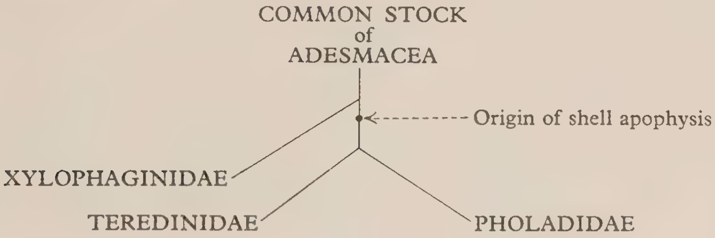
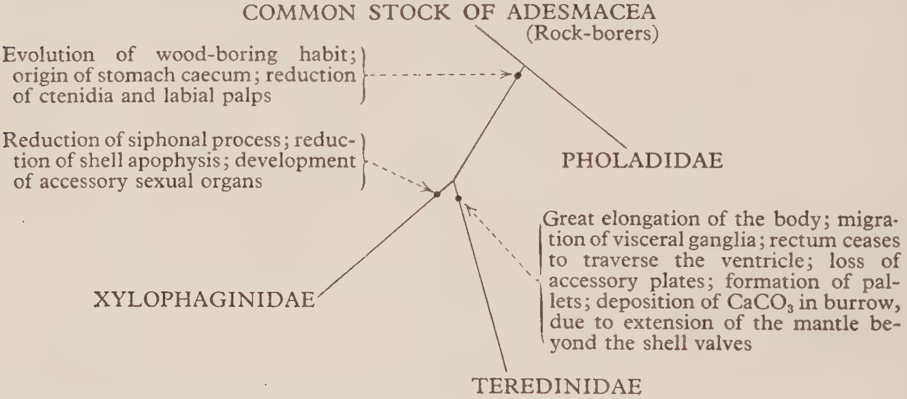


Fig. 15.

The wood-boring stock then divided into the Xylophaginidae and the Teredinidae. In the former the shell apophysis was lost, and the insertion of the pedal retractor muscles moved back to the primitive position; the exhalant siphon was also greatly reduced. The accessory genital organs were evolved. In the Teredinidae, elongation of the body occurred with the accompanying modifications in the anatomy set down in Fig. 16. It has not been possible to make any suggestion as to the reason for the reduction of the exhalant material in the posterior end of the burrow.

The reduction of the ctenidium in the Teredinidae and in the Xylophaginidae to a single demibranch, and the complete loss of ctenidial sorting mechanisms seem best related to the absence of turbidity in the open sea. The same explanation may be offered for the reduction of the labial palps. Where there is no danger of the mantle cavity or the alimentary canal becoming filled with sediment it is not surprising to find a corresponding reduction in the ciliary sorting and cleansing mechanisms. It is recorded that *Teredo megotara* usually occurs in driftwood (Calman, 1919) and that *T. navalis* is intolerant of estuarine water. In these species the reduction of the labial palps has been carried to an extreme; in *Xylophaga* also, the palps are greatly reduced. *Teredo norvegica*, however, is the most common shipworm in European waters on piers or lock gates where the turbidity may be high. In this species the labial palps show least reduction.



Evolution of wood-boring habit; origin of stomach caecum; reduction of ctenidia and labial palps

Reduction of siphonal process; reduction of shell apophysis; development of accessory sexual organs

Great elongation of the body; migration of visceral ganglia; rectum ceases to traverse the ventricle; loss of accessory plates; formation of pallets; deposition of $CaCO_3$ in burrow, due to extension of the mantle beyond the shell valves

Fig. 16.

The caecum of *Teredo* and of *Xylophaga* has been considered to be a mechanical contrivance, storing wood fragments while they are accumulating rapidly during boring operations, so that a strain is not placed upon the remainder of the alimentary canal. It is here considered that the caecum is also a contrivance to convert the spasmodic arrival of large quantities of material into a slow steady stream through the intestine. In other words, the caecum is as much a constant level reservoir as a safety valve.

The evidence obtained from the single experiment upon the digestive enzymes of *Xylophaga* suggests that this animal may be able to digest certain constituents of wood. Further evidence is required on this matter, and it must be borne in mind that *Xylophaga* can live in the absence of wood. In the shipworms, as noted by Nelson (1918) and Lazier (1924), the style sac and crystalline style are small when compared with those of rock-boring lamellibranchs of a similar size (see Figs. 7*b*, 8, SS, CS). This is also true of *Xylophaga*. If the wood-boring lamellibranchs are all capable of digesting cellulose or hemicellulose the animal may be largely independent of digestive enzymes from the style.

If *Xylophaga* is capable of digesting wood it is important to know how this is carried out. Potts has shown that regions of the digestive diverticula, specialized for the intracellular digestion of wood fragments, do not occur in *Xylophaga*; it is possible that wood is digested by the bacteria which have occasionally been found in the alimentary canal. It would be interesting to know whether bacteria are also present in the caecum of *Teredo*. It could be postulated that wood digestion by bacteria is primitive and its intra-cellular digestion in *Teredo* a later development.

Protandric hermaphroditism has now been demonstrated in all genera of wood-boring lamellibranchs. This condition, however, has not been recorded for any member of the Pholadidae. Incubation of larvae, which is known to occur in *T. navalis* (see Calman, 1919), has not been recorded for *Xylophaga*, but owing to the exceedingly heavy colonization of driftwood by small individuals in the samples obtained, it seems highly probable that the larvae are incubated until a late stage of development. Examination of a sample collected in the summer would in all probability settle this point. Whenever the establishment of young individuals upon a suitable substrate is a question of hazard modifications may be expected in the reproductive system. Such is the case in fluviatile, or commensal lamellibranchs, where the larvae are incubated in the supra-branchial cavity. *Xylophaga* possesses certain modifications which render self-fertilization possible. These modifications are believed to be without parallel in the Lamellibranchia. A pair of vesiculae seminales store spermatozoa formed during the male phase, and an accessory genital organ in the supra-branchial cavity is thought to assist in this process by directing the sperm by ciliary activity to the openings of these organs. Grobben (1892) recorded in *Cuspidaria* a pair of internal glandular organs associated with the genital apertures in male specimens. These organs are of unknown function. With this exception, *Xylophaga* is apparently unique amongst the Lamellibranchia in the possession of accessory genital organs functional only in the male phase. These modifications and developments in the reproductive system, if, as appears probable, they render the fertilization of ova more likely, will be of the greatest survival value.

SUMMARY

The exhalant siphon of *Xylophaga* is greatly reduced and its opening is situated within the burrow. Faeces accumulate in the posterior end of the burrow in a compact mass.

The ctenidia and labial palps of *Teredo*, *Bankia* and *Xylophaga* are modified and reduced, unlike those of the Pholadidae. The ctenidia are flat and homorhabdic and possess no ciliary sorting mechanisms; they consist of the outer demibranch only.

The ctenidia of *Xylophaga* are composed of a direct lamella; those of *Teredo* and *Bankia* are divided into anterior and posterior portions connected by a branchial groove which is an extension of the marginal food groove of the gill. The number of filaments in the anterior portion of the gill varies according to the species, and is of assistance in identification.

The labial palps of *Teredo norvegica* are least reduced; in *Xylophaga dorsalis* reduction is greater, though selection of food material is still exercised. The greatest reduction has occurred in *Teredo megotara* and in *T. navalis* where there is no selection.

The ciliated mantle tracts in *Teredo* and in *Xylophaga* play an important part in limiting the quantity of food particles passed from the ctenidia to the mouth.

The alimentary canal of *Xylophaga* is modified in a similar way to that of *Teredo* for the passage through it of large quantities of material. The stomach is enlarged and possesses a conspicuous caecum; the style sac is reduced; there is no typhlosole; the rectum passes through the ventricle.

The caecum probably serves both as a safety valve and as a mechanism to ensure a slow steady stream of wood particles through the intestine. Bacteria and phagocytes may occur in the lumen.

Xylophaga may derive some nourishment from wood, but there is no region of the digestive diverticula specialized for ingestion of wood particles.

Xylophaga is a protandric hermaphrodite; possibly more than one change in sex occurs.

A pair of vesiculae seminales lies in the suspensory membrane of the ctenidium, close to the openings of the genital ducts. They are filled with spermatozoa during the male phase. A ciliated, glandular organ is present in the supra-branchial cavity during the male phase only. This accessory genital organ probably directs spermatozoa into the vesiculae seminales.

The presence of these two structures renders self-fertilization possible and this may be of great survival value to a species which lives in isolated communities.

It was not determined whether *Xylophaga* incubates larvae in the mantle cavity, but this is regarded as probable.

The relationships of *Xylophaga* are discussed and it is concluded that the genus should be removed from the family Pholadidae and placed in a new family in the same order (Adesmacea) but more nearly related to the Teredinidae than to the Pholadidae. It is proposed to name this family the Xylophaginidae.

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LIST OF ABBREVIATIONS USED IN FIGURES

A	Anus
AA	Anterior adductor
AAA	Accessory anterior adductor
AAO	Anterior aorta
AB	Afferent branchial vein
AC	Anal canal
AF	Anterior pallial fold
AG	Accessory genital organ
AP	Accessory plate
APC	Anterior portion of ctenidium
AS	Auricle of shell
AU	Auricle (of heart)
BG	Branchial groove
C	Caecum
CA	Ctenidial axis
CE	Cut edge of shell
CH	Cephalic hood
CM	Cut edge of mantle
CMG	Ciliated mantle groove
CMT	Ciliated mantle tract
CS	Crystalline style
CT	Ctenidium
DD	Digestive diverticula
DF	Dorsal pallial fold
DL	Descending lamella of ctenidium
DLP	Dorsal labial palp
E	Epithelium (of the siphonal process)
EB	Efferent branchial vein
ES	Exhalant siphon
ESS	Blind end of style sac
F	Foot
FE	Floor of exhalant siphon
FR	Faeces in rectum
G	Gonad tubules
GD	Genital duct
GS	Gastric shield
I	Intestine
IC	Infra-branchial cavity
ID	Inner demibranch
IM	Intucking of mantle
IS	Inhalant siphon
L	Ligament
LA	Lamella (or blade) of accessory genital organ
LO	Lateral oral groove
LP	Labial palps
LV	Lumen of vesicula seminalis
M	Mantle
ME	Mantle edge
MG	Marginal groove
MO	Mouth
O	Oesophagus
OC	Opening of caecum into stomach
OD	Outer demibranch
OF	Opaque disk of foot
OG	Opening of genital duct
PA	Posterior adductor
PAO	Posterior aorta
PE	Peduncle of accessory genital organ
PG	Pedal ganglion
PPC	Posterior portion of ctenidium
R	Rectum
RP	Retractor pedis muscle
RS	Retractor muscles of siphonal process
S	Shell
SA	Shell apophysis
SC	Supra-branchial cavity
SIT	Split in tissues
SM	Suspensory membrane of ctenidium
SP	Dorsal surface of posterior adductor
SS	Style sac
ST	Stomach
V	Ventricle
VA	Ventral adductor
VLP	Ventral labial palp
VM	Visceral mass
VP	Ventral pallial muscle
VS	Ventral articulation of shell
W	Wedge-shaped mass of mucous glands
WS	Wall of stomach
X	Vortex in the ciliary currents on flank of foot

THE BIOLOGY AND TREMATODE PARASITES OF THE GASTROPOD *LITTORINA NERITOIDES* (L.) ON THE PLYMOUTH BREAKWATER

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(Text-figs. 1–11)

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INTRODUCTION

Crevices in rocks about high-water mark are usually regarded as the typical habitat of *Littorina neritoides* (L.). The species was thought to be viviparous until in 1935 Linke and Lebour showed that its egg capsules were pelagic, and the investigation described here was begun primarily to ascertain how these egg capsules reached the sea. The first part of the paper deals with the type of habitat occupied by the adults, and their distribution at different levels. The second part deals with spawning and comprises three sets of observations; first, the state of the gonads was determined by dissections through the year; secondly, field experiments were carried out to find whether there was any downwards migration for spawning; and thirdly, the egg capsules were collected from the plankton for a part of one winter and the following spring, and an attempt is made to show that their occurrence is related to meteorological conditions. The third part of the paper deals briefly with the distribution of size groups in the population, growth rate, and the sex ratio. The fourth part is an account of the occurrence of larval trematodes in the species. Dissections showed that the snails were heavily infected with cercariae, and records were kept of their incidence in order to find out whether they caused sterility, influenced the growth rate, or produced sex reversal.

The Plymouth Breakwater was selected as a starting-point for this investigation, as it is a long stretch where conditions are much more uniform than on the same length of shore. It was originally intended to extend the observations to other localities, but this has been impossible save to a very limited extent; all the work described below was carried out on material collected from the Breakwater except where other places are specifically mentioned.

I am greatly indebted to Dr E. J. Allen, F.R.S., through whose kindness I was able to begin this work; to Dr Kemp, F.R.S., Director of the Plymouth Marine Laboratory, who has given me every facility and encouragement for continuing it; and to the Zoological Society for the use of its table at the Plymouth Laboratory for a month in 1939. Amongst the many people who have helped me I wish particularly to thank Dr Lebour for her constant interest, Dr H. B. Moore for much assistance with field work, Mr G. M. Spooner for his help with statistical data, and Mr G. I. Crawford for reading part of the manuscript. Mr William Searle, of the M.B. *Gammarus*, collected all the samples for me, from May 1937 to June 1938, and I am most grateful to him for much help with field work, particularly in August 1939.

THE PLYMOUTH BREAKWATER HABITAT

The building of the Breakwater was begun in 1812 and completed in 1851; four million tons of native limestone and two and a half million tons of granite facings were used in its construction. It is approximately 1600 m. long, and consists of a central part and two arms of about 330 and 360 m. (see map in *Plymouth Marine Fauna*, 1931). The central part faces 2° west of south, the eastern arm about 15° east of south, and the western arm about 17° west of south. Levels were kindly given to me by the Civil Engineer in Chief at the Admiralty. The top is approximately 13·7 m. wide, and its mean level is 0·76 m. above high-water ordinary spring tides. The northern and southern sides are at slopes of 2 to 1 and 5 to 1, respectively. Numerous small cylindrical holes, which were probably used in hoisting the blocks into position, occur on the surface. They vary in size, but are generally 7–9 cm. across, and 8–15 cm. deep.

Very heavy seas sometimes sweep over the Breakwater during the winter. Twenty-four merchantment were wrecked in the Sound in one storm early in the nineteenth century, and about 30 years ago two 80-ton blocks of concrete lying on the southern face of the Breakwater were skidded across the top near the lighthouse at the west end. The degree of exposure is therefore very considerable and fucoids are sparse on both slopes. The factor for the wave exposure of certain localities may be calculated, according to Moore's formula (1935, p. 80; 1936, p. 66), as "the number of days per 100 days in which any wind blows into the exposed aperture of the locality in question, this opening being the seawards aperture measured at a distance of half a mile". This formula does not seem applicable to the Breakwater. If the aperture is measured at a distance of half a mile it is 180°, and the modifying effect of the land-masses to the east and west is not taken into account. It seems better then that the aperture should be taken from the angle between two lines drawn from the middle of the Breakwater to Reny Rocks and Penlee Point, that is approximately a quadrant facing south-east to south-west. According to unpublished wind roses at the Meteorological Office at South Kensington (observations from 1893 to 1922, Fig. 1), the percentage number of winds blowing through this aperture throughout the year is 33. The mean for October to March, inclusive, is 32, for the summer months 35, and the highest average for a single month 40 for August. But gales are most frequent during the winter months (Fig. 1), and, as Johnson points out (1919, p. 72), the prevailing wind may not be the dominant wind. The foreman of the repairing gang on the Breakwater tells me that the most destructive wind there comes from the south-east. The factors that affect the degree of exposure there are thus very complex and cannot be expressed by a single figure. Direct measurement of the dynamic force of the waves by some type of dynamometer

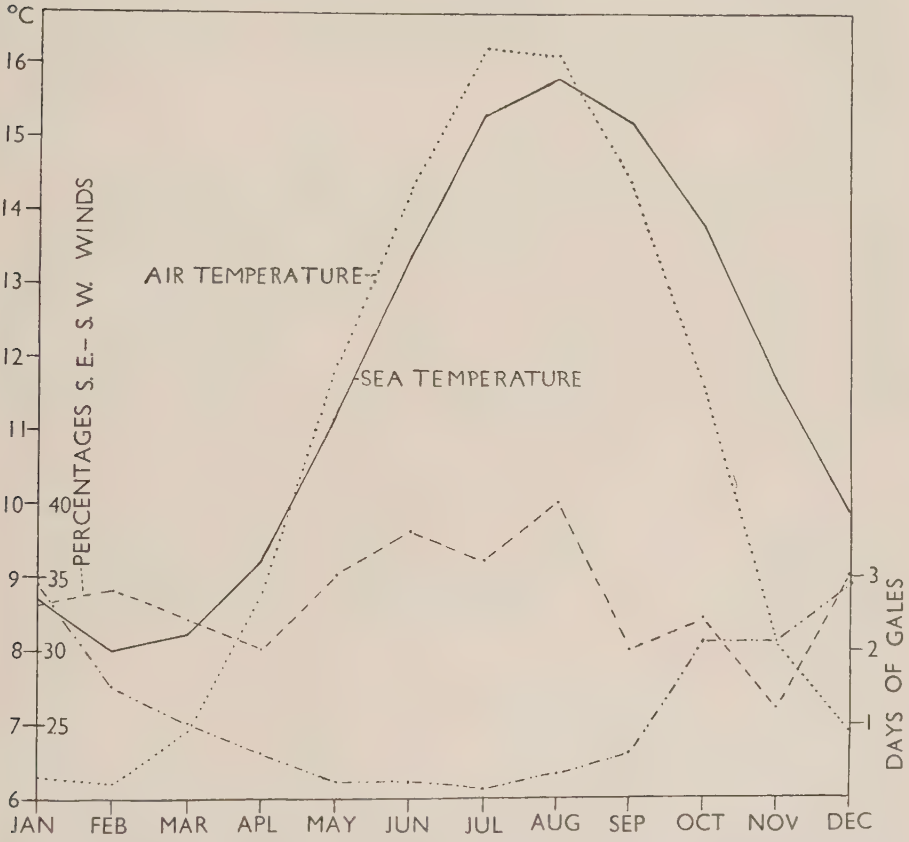

Fig. 1. Sea and air temperatures, percentages of winds blowing from the south east-south west quadrant, and the numbers of days of gales per month in Plymouth Sound.

(Gaillard, 1904) will probably prove to be the most satisfactory method of comparing the exposure of different localities.

The figures for the sea temperatures were kindly supplied by the Plymouth City Meteorologists, Messrs Prigg, Lynden and Ivory. The monthly means are compiled from observations from 1897 to 1932, the temperatures being taken three times weekly at a depth of 6 ft. off the end of Promenade Pier. The air temperatures, for Plymouth Hoe, were supplied by the Meteorological Office at South Kensington. They are compiled from daily means from 1906 to 1935.

ZONATION ON THE BREAKWATER

Two traverses were made across the Breakwater in August 1939, the first, A (Fig. 2), 345 m. west of the midpoint, and the second, B (Fig. 3), 184 m. east of the midpoint. Two surface samples were taken at each level at A, but, owing to limitations of time, only one at each level at B, where there were also fewer stations. Each sample consisted of the total number of snails collected in 100 sq. cm. The population in the pools on the top was estimated from two pools at each of four stations in both traverses; the first station being situated at the north edge, and the others 4·5, 9 and 13·5 m. south of it. The depth of each pool and the volume of water in it were measured and the approximate area of the sides and bottom calculated from the formula $2\pi\,(\sqrt{v/\pi H})\,H$. The results are shown in Table I and Figs. 2 and 3.

TABLE I

Levels of "dry" stations in relation to mean sea-level m.	Traverse A			Traverse B		
		Height of shell			Height of shell	
	No. per 100 sq. cm.	Range mm.	Mean mm.	No. per 100 sq. cm.	Range mm.	Mean mm.
North slope 0·8	0	—	—	0	—	—
1·7	0	—	—	—	—	—
2·2	6	2·0–3·2	2·6	—	—	—
2·6	11·5	2·0–3·2	2·4	61	2·0–4·7	2·7
[E.H.W.S. 2·72]						
Top 3·0 *A*	6·5	2·0–5·9	3·3	27	2·0–4·4	2·6
B	23	2·3–6·2	3·7	37	2·6–6·5	4·3
C	83	2·0–5·9	3·0	14	2·6–7·1	5·0
D	109	2·0–3·8	2·5	56	2·3–4·4	3·1
[E.H.W.S. 2·72]						
South slope 2·6	134	2·0–4·5	2·7	70	2·0–5·0	3·0
2·2	59·9	2·0–3·8	2·4	—	—	—
1·7	27	2·0–3·2	2·3	—	—	—
1·3	6	2·0–2·6	2·3	17·5	2·0–2·9	2·4
0·8	6	2·0–2·6	2·2	—	—	—
[M.S.L. 0]						
−0·1	2	2·0–2·6	2·3	—	—	—
−0·6	0	—	—	—	—	—
"Wet" stations						
Top 3·0 *A*	13·2	2·0–7·7	4·5	8·6	2·0–7·1	3·5
B	54	2·0–6·8	4·6	19·2	2·3–8·0	5·1
C	33·1	2·0–7·4	4·5	33·85	2·9–7·7	5·3
D	20·8	2·0–6·8	3·8	44·2	2·3–6·8	4·1

E.H.W.S. = extreme high-water springs; M.S.L. = mean sea-level.

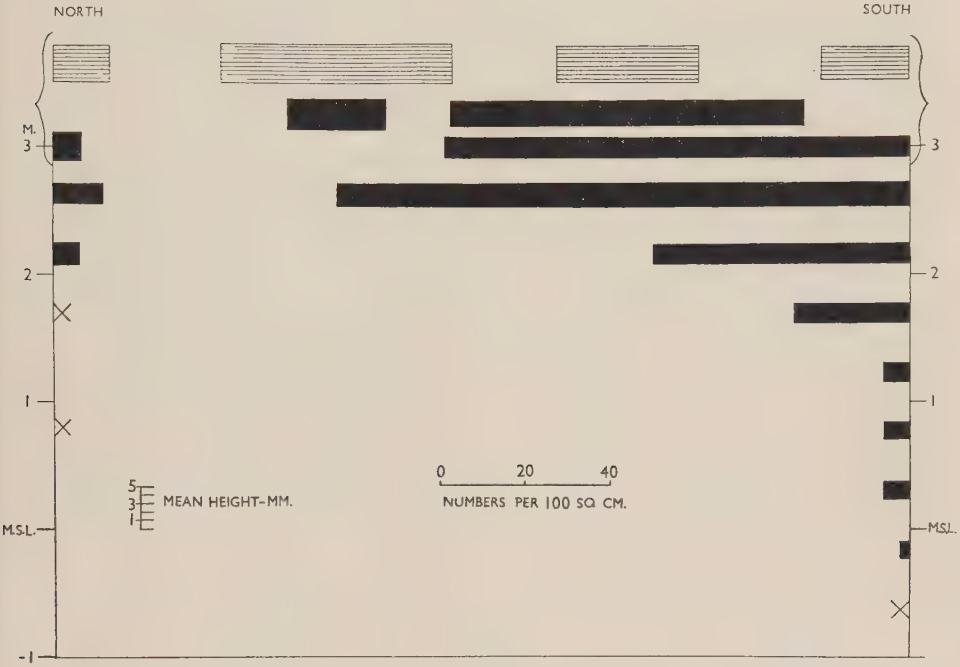

Fig. 2. Numbers of snails per 100 sq. cm. at different levels on the Breakwater in traverse A. Those collected from "dry" stations are shown in solid blocks, and those from the pools in horizontally ruled blocks. The height of the blocks shows the average height of the snails at each station. The eight bracketed samples were all taken across the top of the Breakwater, 3 m. above mean sea-level (M.S.L.).

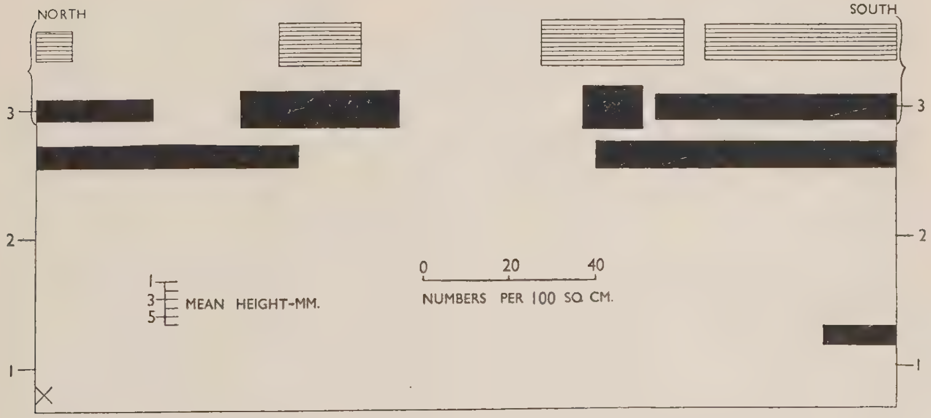

Fig. 3. Numbers of snails per 100 sq. cm. in traverse B. Explanation of blocks as in Fig. 2.

The height of the shell was measured with a pair of sliding callipers with a 0·1 mm. Vernier scale. Snails less than 2 mm. in height are included in the 2 mm. groups owing to the difficulty of measuring them with any degree of accuracy. They rarely occurred except at the lowest levels.

The traverses showed that the snails were most abundant towards the top of the southern slope just below extreme high-water springs, i.e. 2·6 m. above mean sea-level (Fig. 2, Table I). Small snails were found 0·15 m. below mean sea-level, and also occurred at the top of shelter blocks (not included in the traverses) 5 m. above mean sea-level. Colman (1933) found that at Wembury they occupied a zone of only 2·72 m., extending vertically from about 1·25 m. above extreme high-water springs down to mean high-water neaps. The zone occupied on the Breakwater is therefore of considerably greater vertical extent than at Wembury, but it is possible that the small snails at the bottom of the zone were overlooked in the latter locality as they usually shelter amongst the barnacles and are difficult to find. Owing to the high degree of exposure on the Breakwater all the snails are within the splash zone at least during the winter months, when, owing to the heavy swell, the sea washes over the top twice a day at high tide.

RELATION TO ECOLOGICAL FACTORS

Light

The habitats selected are very variable with respect to light. It has already been noted that on the Breakwater the population is most dense almost at the top of the southern slope where the maximum amount of sunlight falls. During the winter the top of the Breakwater is covered with a fairly dense layer of algae, which retains moisture and gives a certain amount of shelter. During the summer, however, algal growth is negligible, and the snails have to endure prolonged exposure to the sunshine. At Tinside, Plymouth, they are common in crevices on the southern slopes, but also underneath overhanging rocks at the entrance to a cave where there is no direct sunlight. They occur in equally exposed and equally shaded situations on Drake's Island, and also at Crantock and Kelsey Head in north Cornwall. Furthermore, on the top of the Breakwater where many of the larger snails live in water in small cylindrical pits, they are equally distributed on all sides of these pits, and at all depths.

Flattely & Walton (1922), discussing the distribution of this species in Cardigan Bay, say that it occurs there in crevices just above high-water mark, which are not exposed to the midday sun, and that the same type of distribution occurs on the Devon coast. Colman (1933), however, found that at Wembury the snails "congregate not only in cracks or crannies where it is damp, but also in hollows which are quite dry and also directly face the sun". He goes on to remark that "they are always very loosely attached to the rock so that one can easily blow them off" and suggests that "the search for hollows and cracks is more an avoidance of the mechanical force of the wind than of the desiccation caused by it". He refers to Fränkel's (1927) work on taxes in this species, which was found to be negatively geotactic, negatively phototactic when above a horizontal substratum, but positively phototactic when hanging from a horizontal ceiling. Colman concludes by saying that the resultant of these reactions would make the animals congregate round the entrance to fissures and in hollows, where, in fact, they are found in nature. Actually, Fränkel qualifies his remarks. He says (free translation): "However, this change in phototactic response occurs only in water. In air *Littorina* is negatively phototactic even when hanging from horizontal ceilings."

That these responses are highly modified under certain conditions in the field is obvious from the distribution of the snails on the Breakwater, and it seems likely that the concentration on the exposed sunlit slopes there may prove indicative of the general distribution of the species. The following observation by Fischer-Piette (1936, p. 248) is of considerable interest in this respect: "Il est curieux de voir cette espèce mieux réprésentée sur la côte anglaise que sur la côte française de la manche, étant donné que, par ailleurs, elle est particulièrement bien développée dans les régions plus méridionales telles que la Loire Inférieure, la côte basque, la Méditerranée."

Gravity

The distribution of the snails at different levels is set out in Figs. 2 and 3 and in Table I, and shows clearly that the smallest individuals are commonest at the lower levels and the larger ones at the upper levels.

The average size of the snails in the samples on the northern and southern slopes varied from 2·2 to 3·0 mm.; at the surface stations across the top from 2·5 to 5·0 mm.; and in the pools across the top (the "wet" stations) from 3·8 to 5·3 mm. The largest snail found on the slopes was 5·0 mm. high, and on the top 7·7 mm. Large snails occur in the deep crevices between the blocks where the water drains away as the tide goes down, but no estimate was made of their size or of the density of the population in such situations.

The distribution of the size groups suggests that the metamorphosing larvae settle in the *Chthamalus-Balanus* zone and that there is a gradual migration towards the higher and drier levels generally recognized as their typical habitat. Further evidence of upward migration in the field is given in the section dealing with spawning (p. 53, Table II).

In the laboratory when the snails are placed in water in a finger bowl they immediately crawl to the top; this reaction is sufficiently constant to be an efficient method of separating them from *Littorina saxatilis*, which is common in the lower part of the zone occupied by *L. neritoides*.

In the field this negatively geotactic response is sometimes reversed. Throughout most of the year the snails are evenly distributed on the vertical sides of the pits on top of the Breakwater. During the winter there is usually a swell sufficiently heavy to sweep over the top at high water, so that the water in the holes is constantly renewed. Under hot and dry conditions when tides are low this does not happen, and evaporation causes a considerable increase in salinity. The snails then crawl up the sides and congregate in dense masses between the water level and the lip of the holes. When the water has been renewed they crawl down again. If these snails are collected and placed in water in glass finger bowls they give a negatively geotactic response and immediately crawl to the top of the bowls.

Moisture

A certain amount of moisture is essential for the survival of *L. neritoides*. First, the snails feed on minute algae that grow on the rocks, and they move about and feed only when the surface is moist; they are as active during rain as when they are submerged in sea water. Secondly, it is most unlikely that spawning occurs except when the snails are submerged; evidence supporting this is given later on.

That they live in very dry situations has long been known (Jeffreys, 1865; Forbes & Hanley, 1853). They are able to survive in situations at the top of their zone where they may not be reached by spray for months at a time. Patanè (1933) found that they were able to survive absence of moisture for at least 5 months, and that they regained their activity within a few minutes of being placed in sea water.

On the other hand, observations in recent years have shown that not only do they sometimes occur in shallow pools (Lebour, 1935), but that they are able to live in places where they are permanently submerged. When they are placed with small pieces of rock in sea water in finger bowls most of the snails remain on the projecting pieces, but a few of them crawl beneath the water and remain there. Dr H. B. Moore kindly gave me a few living specimens that he collected from old piles lying beside the pier at Plymouth, which had been submerged for at least a year, and ripe sex cells were present in both a male and a female that I dissected. Mr G. M. Spooner first drew my attention to the fact that on the Breakwater many of the snails live in water. When individuals collected from the pools there were marked with paint and replaced, 95 of 103 recovered a year later were still in the pools. The density of the population in water in the holes is very similar to that on the exposed rock surfaces (Figs. 2, 3, Table I); the most noticeable difference between the snails in the two situations is that the largest ones are more numerous in the holes. This is significant in view of the fact that no snails live in water in the holes on the slopes save just at the upper edge, so that none occurs in pools at levels where the smaller sizes predominate. Yet under laboratory conditions small snails are much less able to withstand emersion than are the large ones.

It is probable that the holes on top are inhabited mainly on account of the shelter from heavy seas that they afford and that the need for moisture plays little part in their selection. No other locality has been found in the Plymouth area where many snails live permanently in water. Fischer-Piette (1932) states that at Cap Martin the species is abundant in holes above high-water mark. He notes particularly that there they are always to be found in the water despite great variation in the salinity.

One final point of interest with reference to moisture may be mentioned here. Fischer *et al.* (1933) have shown that the consumption of oxygen by *L. neritoides* is 5–6 times higher in water than in air.

Shelter

A suitable settling ground for the metamorphosing larvae of *L. neritoides* is afforded by populations of *Chthamalus stellatus* and *Balanus balanoides*. The presence of abundant fucoids appears to be inimical to the larvae. Hatton & Fischer-Piette (1932) state that on rocky shores the absence of these algae indicates high wave exposure, and that their abundance is proportionate to the degree of shelter available.

At the head of Crantock Bay, north Cornwall, cliffs rise from a sandy beach which is exposed for several hours at low tide. *Littorina neritoides* is not abundant there except on rocks where barnacles are present. Fucoids are absent. The mollusc is scarce on the west side of the bay where there are slopes of broken rock and these algae are fairly abundant. At the end of June 1939, a similar type of distribution was found in Northern Ireland. East of Whitepark Bay in County Antrim there is a limestone cliff at the foot of which the snails are so scarce that in 5 min. search only six were collected. A shelf of low boulders extends seawards for 20–30 yards from the foot of the cliff, and is covered with a mat of fucoids so dense that it is extremely difficult to walk across it. At the edge of the shelf there are larger limestone boulders, 2–4 ft. high, which are bare of *Fucus*, and beyond them the water deepens and *Himanthalia* and *Laminaria* are abundant. On these larger boulders there is a dense population of the snails, and *Balanus balanoides* (kindly identified by Dr H. B. Moore) is fairly common. *Littorina neritoides* was also abundant and very conspicuous on finely pitted white limestone boulders in a very wave-beaten bay, Larry Ban, west of Carrick-a-rede, where there was no appreciable algal growth above the *Laminaria* zone. It was, however, scarce on smooth-surfaced limestone rocks in a very exposed position east of Ballintoy Harbour. Barnacles are common and fucoids very sparse on the Plymouth Breakwater where the snails are very abundant.

Dr H. B. Moore has very kindly supplied me with notes (Appendix) on some localities in Scotland where he has searched for *Littorina neritoides*; these notes support my conclusions that the presence of the species is positively correlated with a high wave exposure and the presence of barnacles, and negatively correlated with abundant fucoids.

Kitching (1935) says "the absence of *Balanus balanoides* from the immediate neighbourhood of fucoids is almost certainly due to the rubbing of the fronds, which might either prevent the larvae from settling, or damage the young barnacles", and Moore & Kitching (1939, p. 525) consider that the distribution of *Chthamalus* is affected in the same way.

It has already been observed that the small snails are able to survive emersion for a much shorter period than are the large ones. They are negatively geotactic and gradually migrate towards the top of the zone, apparently in their search for shelter. The large snails are able to withstand very dry conditions. Where, on the Breakwater, the highest and driest level available does not afford the maximum amount of protection some snails have become positively geotactic, and live permanently in water.

SPAWNING

The Spawning Season and its Duration

Samples of *Littorina nerotoides* were dissected at different times of the year and the state of the gonads determined. Males are defined as *unripe* when the spermatozoa were present only in bundles, *ripe* when active spermatozoa were present either alone or with bundles, and *spent* when no spermatozoa, either alone or in bundles, could be distinguished. Females are defined as *unripe* when oocytes alone or with all stages of developing eggs except ripe ones were present, as *ripe* when ripe eggs were present perhaps in addition to large numbers of unripe ones and oocytes, and as *spent* when there were no eggs, or very few, either ripe or unripe. It appears that infection with some trematode parasites produces sterility, so that individuals in which unencysted cercariae occurred are not considered here although they formed a considerable part of some samples. The snails examined were all collected from the top of the Breakwater, and most of them were taken from the pools or their immediate vicinity.

In samples containing 39, 74 and 62 normal males, collected on April 30, June 10 and July 30 1937, respectively, 85, 100 and 79 % were spent, but by mid-September active spermatozoa were present in 90 % of those examined (Fig. 4). Nearly 100 % were ripe in samples examined in October, December and January. In early March a few spent males were found, and by May and June their numbers had risen to 67 and 93 %, respectively.

Egg production is at a minimum during June, July and August (Fig. 5). In mid-September 1937, more than half the females examined contained oocytes or unripe eggs. Ripe eggs were found in 60 % of those examined in December, and in 50–60 % in January, March and May 1938. Although the percentage of ripe females did not exceed 63 in any sample examined in 1937–8, it rose to 73 and 76 in February and March 1936.

The spawning period thus lasts altogether for about 8 months, that is, from September to April. The sea temperature (Fig. 1) drops from 15·2 to 8·0° C. and rises again to 9·2° C. during this period; the corresponding figures for the air being 14·4, 6·2 and 8·8° C.

Linke (1935), working on material from Rovigno and Majorca, says that the maximum development of the whole genital apparatus in this species is

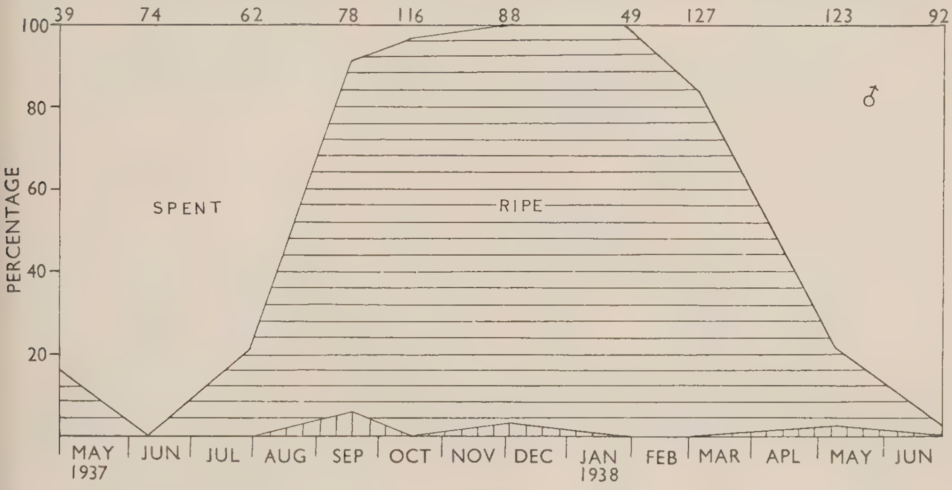

Fig. 4. Percentages of unripe, ripe, and spent males in samples examined every 4–8 weeks from May 1937 to June 1938. The upper figures show the numbers of snails in each sample.

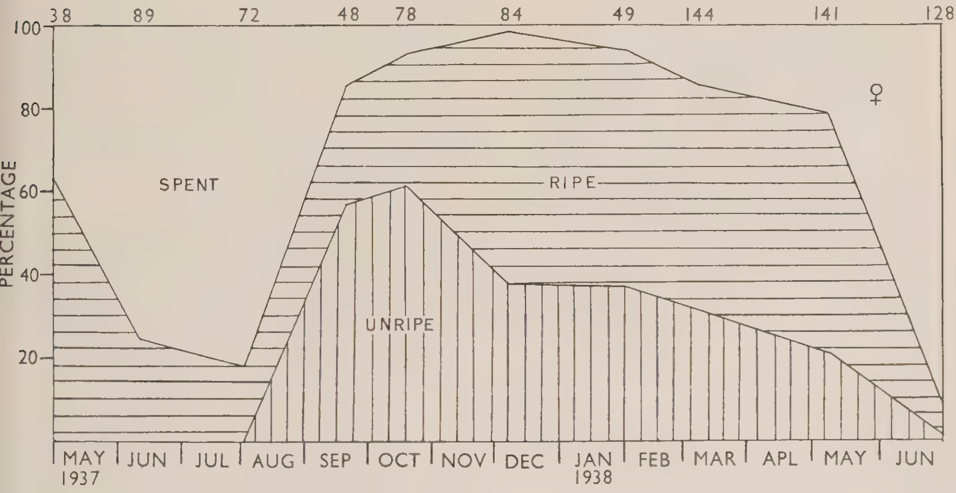

Fig. 5. Percentages of unripe, ripe, and spent females as in Fig. 4.

attained in the spring, but he does not give the size of the samples with which he worked.

An interesting point that emerged from the above dissections is the difference of more than two months in the maximum production of ripe sperms and ova. Moore (1937, p. 733) found that in *L. littorea* the males were ripe about a month earlier than the females, and that the breeding season of this species in the Plymouth area lasted from November to June. *L. saxatilis* breeds all the year round (Lebour, 1937, p. 127). In other localities *L. littoralis* usually breeds from the spring to the autumn (Pelseneer, 1935, p. 452; Linke, 1933), but there is a considerable fluctuation, depending on the temperature, in the breeding period of this species.

Experiments on possible Spawning Migration

Field experiments on migration were carried out by marking the snails with a cellulose paint. If conditions permit the paint should be renewed after 6 months, but this was not actually done. Although a certain amount of wear occurred, some marked snails were conspicuous even after 12 months on the Breakwater: on other snails so little paint was left that they were very difficult to find. This applies particularly to the smaller sizes. Sometimes the snails were painted in situ; at other times they were brought into the laboratory, painted and replaced on the rocks the following day, or sometimes later.

On December 13 1935, two lots of 50 and 200 snails on the rocks at Tinside, Plymouth, were marked in situ with red paint. They occupied zones the lower limits of which were (A) 0·14 and (B) 0·16 m. below M.H.W.S. A month later 44 and 192 individuals respectively were recovered, and only one of these was found below the original zone. On January 24, 222 of these marked snails were placed in a horizontal cleft about 1 m. below the levels A and B. A fortnight later none could be found below these levels. The snails had crawled at least a metre up the rock face, and although they were not counted the marked population was approximately as dense as previously.

On Drake's Island, in Plymouth Sound, in an exposed position above some rocks on the shore, there is a rectangular concrete block approximately 77 cm. high, and 45 by 46 cm. broad; the top of the block is 2·74 m. above mean sea-level. The aspect is roughly south-south-east by north-north-west. On February 4 1936 the west-south-west face was divided into four equal horizontal zones. In the top one, A, 100 snails were painted red, and in the bottom one, D, 100 were painted yellow. No snails were painted in the intermediate zones, B and C. The positions of the snails recovered after 1, 2 and 12 months are shown in Table II. These recoveries show that there is a pronounced tendency for the snails to move upwards, and that only a very small proportion of them move more than a few centimetres in the opposite direction.

In a more sheltered area on Drake's Island where, on January 27 1936, 100 snails were marked with red paint, 67, 48, 49, 47 and 22, respectively, were recovered at their original level when recounts were made on February 4, March 5, April 27 and August 5 1936, and in February 1937. Whenever recounts were made the surrounding rock surfaces were carefully examined, but although the red paint made the snails very conspicuous they were found only once or twice at more than a few centimetres from their original situation.

TABLE II. RECOVERIES OF MARKED SNAILS ON CONCRETE BLOCK, DRAKE'S ISLAND

Date	Colour	No. recovered	Percentage in zones		
			A	B and C	D
4. ii. 36	Red	—	100	0	0
5. iii. 36	„	77	92·2	7·8	0
5. iv. 36	„	51	78·5	19·6	1·9
– ii. 37	„	5	100	0	0
4. ii. 36	Yellow	—	0	0	100
5. iii. 36	„	69	0	79·8	20·2
5. iv. 36	„	44	25·0	52·3	22·7
– ii. 37	„	13	100	0	0

The results of these experiments make it unlikely that there is any downwards migration for spawning as suggested by Lebour (1935, p. 375); and Jeffreys (1863, p. 354) says of the snails, "they have never been observed to go to the sea when the tide comes in".

Occurrence of Egg Capsules

In order to determine when the egg capsules were present in the plankton tow-nettings were examined daily, as far as possible, from November 29 1935 to May 1 1936. The capsules were fairly common at times during the winter and spring in tow-nettings taken by the M.B. *Gammarus* in the Sound, but occurred so rarely in those taken by the S.S. *Salpa* beyond the Sound that the latter were not examined after February 4 1936. The internal diameter of the hoop supporting the mouth of the tow-nets was 45 cm. The capsules, which vary at the height of the breeding season from 0·162 to 0·225 mm. in diameter with an average of 0·205 mm., were not taken in the coarse tow-nets, with a mesh of 26 strands of silk to the inch (25·4 mm.), and very rarely in the medium, 50 strands to the inch, but were commonest in the fine and very fine nets with 100 and 180 strands to the inch, respectively. At first the "*Gammarus*" nets were arranged so that the very fine net was at the surface, the fine at a depth of 2 fathoms, and the medium at 6–8 fathoms. Most of the capsules were then taken at 2 fathoms. The medium net was replaced in March by the very fine net, and capsules were then taken at 6–8 as well as at 2 fathoms. It is obvious that the numbers of capsules collected from the tow-nettings, which were taken in several different places in the Sound and independently of the state of the tide, are not strictly comparable amongst themselves and have no direct relation with samples which were collected from the Breakwater. (In the latter situation water was pipetted from the bottom of seventy holes and about two breffits were filled at each sampling.)

The capsules were found to sink at the rate of 150 mm. in 109–203 and 271–514 sec. at temperatures of 15·5 and 10·0° C. Linke (1935) observed that they sink in still water but tend to remain suspended when it is agitated. This property was useful in separating them from the tow-nettings, which were strained through coarse muslin into a cylinder and left to settle for about half an hour. Most of the water was then gently siphoned off, and the capsules collected from the residue.

The total number of capsules collected, together with some of the factors that may influence spawning, are shown in Fig. 6. If no downwards migration takes place the possible immersion of the snails living above extreme high-water springs would depend on the phase of the moon and the corresponding tides, the direction and force of the wind, and the state of the sea. In Fig. 6 full and new moons are shown by white and black circles, respectively; the predicted tidal heights are given, and those more than 15 ft. above Admiralty Datum are blacked in; the state of the sea is scaled according to the meteorological charts issued by the Air Ministry; onshore and offshore winds tending to raise and depress the predicted tidal levels are shown by arrows pointing upwards and downwards respectively, and where the force of the wind exceeded 3 on the Beaufort scale double barbs have been used. The solid columns show the numbers of egg capsules collected from the tow-nettings in the Sound, and those which are cross-hatched the numbers from the Breakwater samples. The blanks in the base-line show the days when no samples were taken.

Notes on the stage of development of the capsules in some of the samples are given in Table III.

TABLE III. NOTES ON THE STAGES OF DEVELOPMENT OF SOME OF THE EGGS COLLECTED FROM THE TOW-NETTINGS AND BREAKWATER SAMPLES

Date	Locality	No. capsules	Remarks
9. iii. 36	Jennycliff Bay	62	All were undergoing the first or second cleavage
10. iii. 36	Breakwater	75	Most of these had not completed the first cleavage
19. iii. 36	,,	5	All were in different stages of development
23. iii. 36	Jennycliff Bay	52	Embryos with 2–32 cells were present
24. iv. 36	Breakwater	800	Many had not begun to segment and none had passed the first cleavage
31. iv. 36	Jennycliff Bay	46	At a depth of 6–8 fathoms there were 31 embryos of 32–64 cells, and at 2 fathoms 15 with 2–4 cells. Some of the eggs were abnormally small, 0·126–0·144 mm. in diameter, and were segmenting irregularly
1. v. 36	Breakwater	38	There were 2 embryos with 4 cells, 23 with 2 cells, and 14 in which segmentation had not begun

In the five months during which samples were taken there were five periods when more than 50 egg capsules per set of samples were obtained, and four of these coincided with sets of high tides. The apparently exceptional sample,

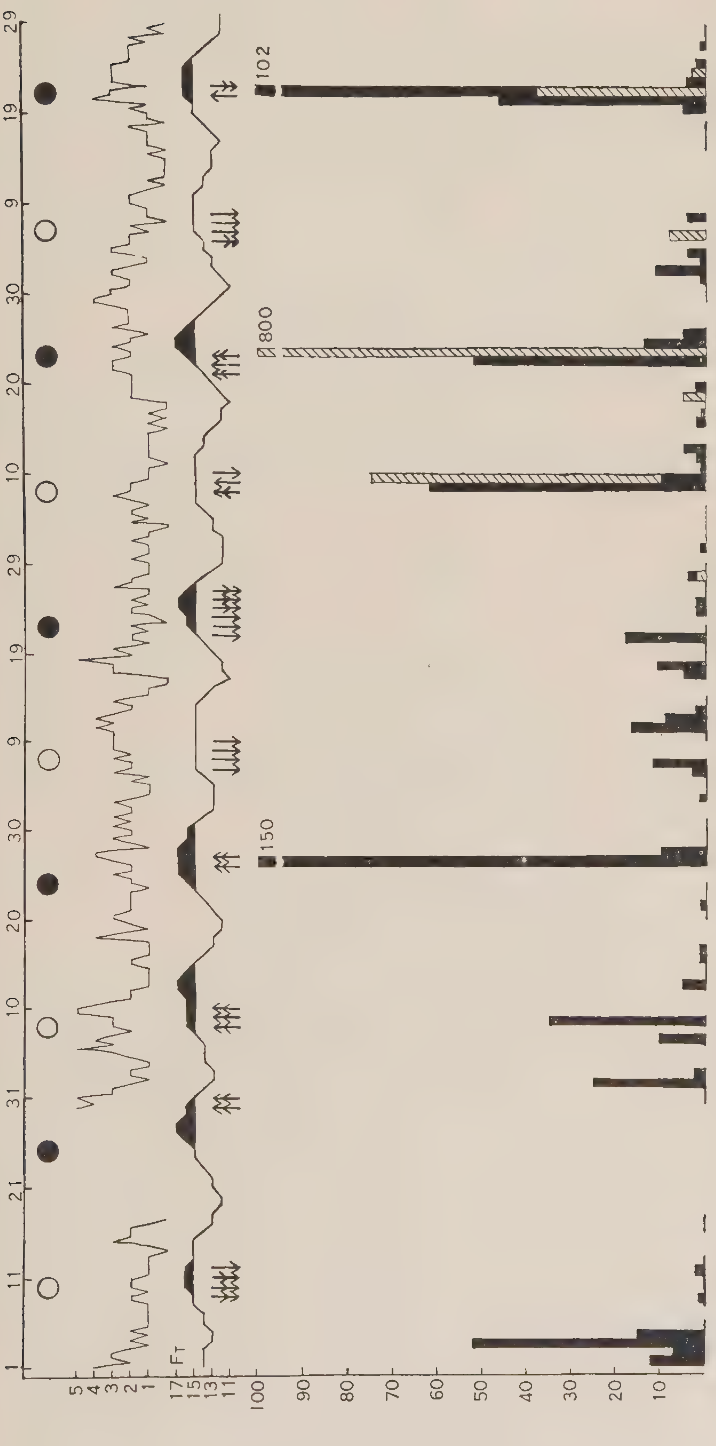

Fig. 6. Abundance of egg capsules of *Littorina neritoides* together with factors affecting the height of the tides and the moisture of the splash zone. The state of the moon is indicated by open and black circles. The upper continuous line shows the state of the sea. The lower continuous line shows the predicted height above Admiralty Datum of high tide, the highest for each day being plotted; heights above 15 ft. are blocked in. The arrows indicate onshore (upwards) and offshore (downwards) winds. For details see text, p. 54. The solid columns show the numbers of egg capsules collected from tow-nettings in the Sound; the cross-hatched columns show numbers collected from Plymouth Breakwater. Blanks in the base line indicate days when no observations were made.

taken in Cawsand Bay at the beginning of December, contained a large proportion of capsules that were sufficiently old to have been spawned during the previous high tides. The eggs hatch in 7–8 days in the laboratory. During five other sets of high tides the capsules were not found in any quantity, but it is probable that the prevailing meteorological conditions lowered the predicted tidal levels, and prevented spawning except in those adults living in the lower part of their zone. When the water in the Sound is calm, as often happens when there is a light wind from the north, even if the tide were sufficiently high to submerge those in the upper part of the zone it is probable that the capsules are not readily dispersed and are therefore less likely to be collected in the tow-nettings.

The strongest indication of the presence of a fortnightly rhythm in the spawning habits of this species is shown by the Breakwater samples. Although many of the snails there live permanently in water, egg capsules were abundant in only three out of seven sets of samples that were examined, and these three were taken when the moon was new or full. This is of particular interest, as during the winter and spring, when breeding takes place, the sea usually sweeps over the Breakwater twice a day. In these samples, as well as in the tow-nettings, when the capsules were plentiful they were usually at the same stage of development. Full notes on the incidence of these stages were not kept, but a few are given in Table III. They suggest that the onset of major spawning periods is determined by some stimulus in addition to immersion. Lunar periodicity has been shown to occur in many other species of Molluscs (Pelseneer, 1935).

Linke (1935) observes that in the spring the egg capsules of *Littorina neritoides* were fairly abundant in the neritic plankton in the Mediterranean, but he does not refer to the presence of any spawning rhythm there.

Composition of the Population

Incidence of the Size Groups

The material for this part of the investigation was all collected from the Breakwater. The 1936 samples included snails from the southern slope, so that there is a larger proportion of small snails in these than in the 1938 samples, which were all taken from the top and mostly from the pools or their immediate vicinity. The snails were measured with sliding callipers with a 0·1 mm. Vernier scale, and are grouped in 0·3 mm. series (Fig. 7).

The largest sample, measured in July 1938, consisted of 7796 snails; those averaging 4·1, 5·0 and 5·9 mm. in height occurred with the greatest frequency. Snails of 5·0 and 5·9 mm. also predominated in smaller samples collected in March and May 1938, and were conspicuous in the 1936 samples. The 5·9 mm. group is less obvious in the June 1938 sample, where the sizes were more evenly distributed, but predominated in the October sample. Snails in the 4·1 mm. group, so conspicuous in the July sample, are noticeable in only one

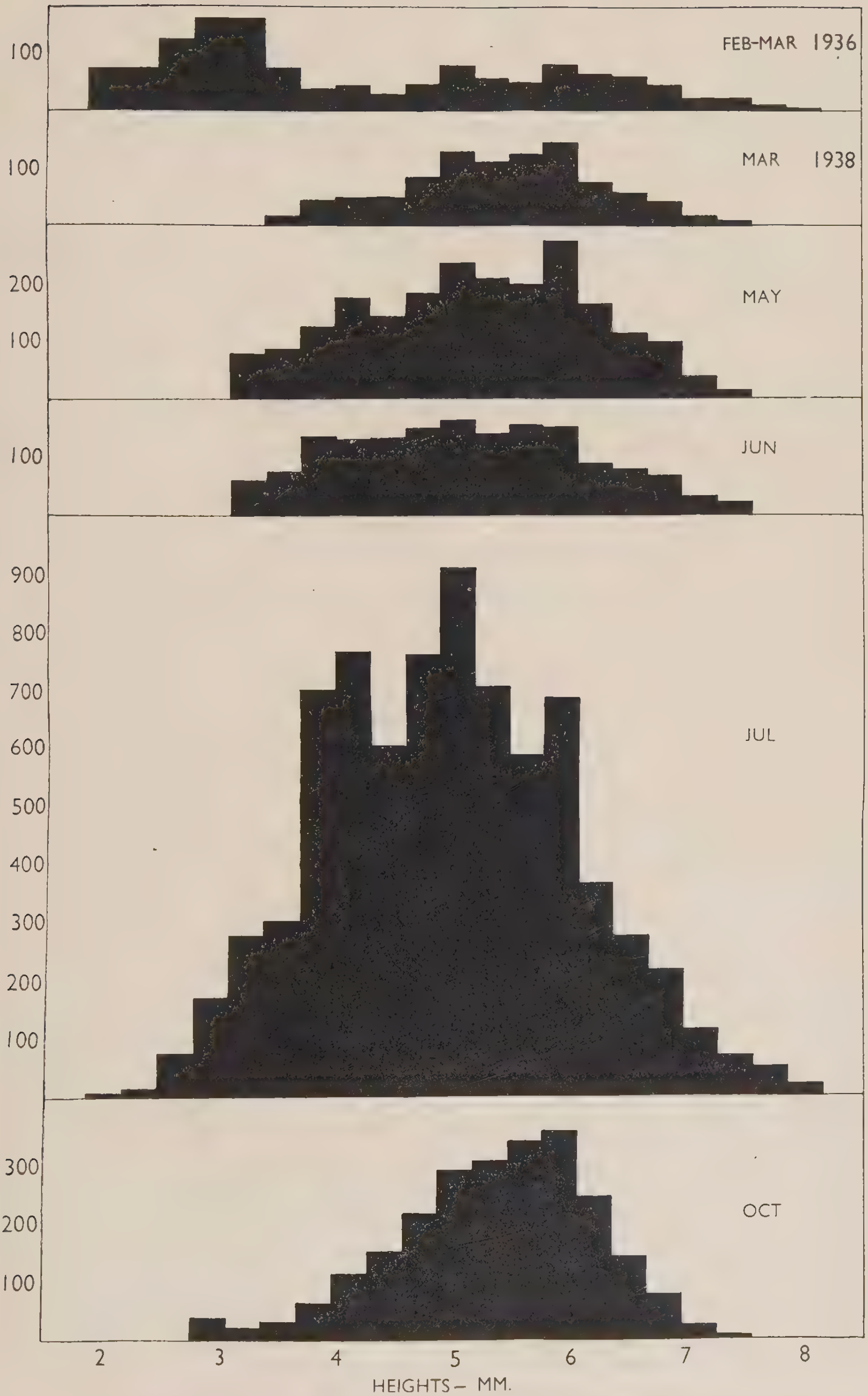

Fig. 7. Numbers of snails in 0·3 mm. size groups in random samples collected from the Breakwater in February–March 1936, and from March to October 1938.

other sample, that of May 1938. In spite of these variations in the incidence of the size groups there appears to be a tendency for the larger snails to fall into size groups separated by approximately 1 mm., but whether these actually represent successive annual broods remains uncertain.

Growth Rate and Sex Ratio

Of 2107 measured snails in five selected size groups, marked with red and yellow paint and replaced on the Breakwater in July 1938, only 102 were recovered a year later. The results of this experiment are shown in Table IV. It will be seen that the growth-rate decreases with age, and is very slow in snails of 6·0 mm. or more. It is probable that those of 8–10 mm. are many years old. The largest one found was 10·4 mm. high. It has been deposited in the British Museum of Natural History.

TABLE IV. GROWTH-RATE AND PARASITES IN MARKED MATERIAL, JULY 1938 TO AUGUST 1939

Height mm.	No. snails marked	No. snails recovered	No. males	Average increase in height mm.	No. infected with Cercaria B and C
3·9–4·0	545	1	0	0·6	0
4·9–5·0	732	38	7	0·48	22
5·8–5·9	448	26	5	0·26	17
6·0	238	9	2	0·14	8
6·8–6·9	144	28	2	0·06	26

The proportion of males is significantly higher in the smaller size groups than in the larger ones (Fig. 8), and the ratio is similar even when only unparasitized ones are considered. This may be due to a differential growth-rate such as Moore (1937) found in *L. littorea*, where the growth-rate is greater in females than in males.

Moore found that on Drake's Island *L. littorea* reached a height of about 14 mm. in the winter of its first year, and 17·4, 22·4, 25·4, and 27·3 mm., respectively, in the second, third, fourth and fifth years. It may grow to a height of 36 mm., that is, about two and a half times the height reached in the first year. In *L. neritoides* the results obtained indicate a comparable steady decrease in the growth-rate. It can also be said that the values of annual increments were such that growth must normally extend over several years.

It has to be noted that many of the snails in this experiment were found to harbour cercarial infections of trematode parasites (dealt with on pp. 59–64). Since Rothschild (1936, 1938*b*) has found that such infections stimulate growth in *Peringia ulvae*, their presence cannot be ignored when the question of growth-rate is being considered. The number of snails infected is therefore included amongst the data in Table IV. The proportion of infected specimens increases with the size of the snail, until in the largest group only two out of the 28 were found to be free of the parasites. The data as they stand do not, on first inspection, suggest that the parasites stimulate growth; but no conclusion can be drawn, since the real information required, namely comparison of growth-rates of infected and *uninfected specimens of the same initial size*, is not available.

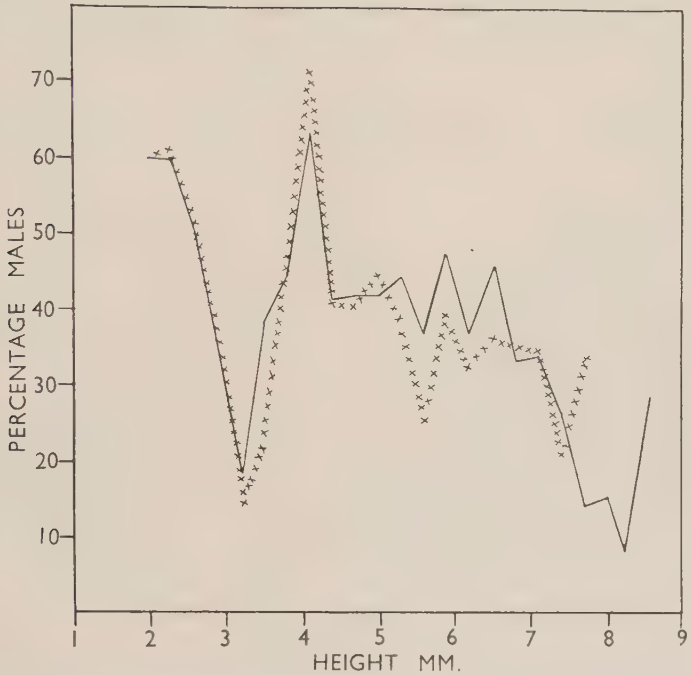

Fig. 8. Percentages of males in 0·3 mm. size groups. The continuous line shows the percentage in parasitized and unparasitized snails, and the crossed line the percentage in unparasitized snails only.

Trematode Parasites

Total Percentage Parasitism

The incidence of trematode parasites was recorded in the snails used for determining the state of the gonads at different times of the year (p. 50), comprising a total of 1270 males and 1779 females. A few samples from localities other than the Breakwater were also examined (Table VI, p. 64).

Three larval trematodes occur fairly frequently in *L. neritoides* on the Breakwater. Their systematics have not been investigated, and throughout this paper they are referred to by letters.

Infections with Metacercaria A, and Cercaria B and C occurred throughout the year, but were significantly higher in the sample examined in July 1937 (Table V) than in any other examined between April 1937 and June 1938.

The percentage of parasitism with all species rose steadily from 3·3 in snails averaging 2·0 mm. in height, to 99·8 in those of 8·8 mm. and over. The relative numbers of cercariae, both free and encysted, in the different size groups are shown in Fig. 9.

TABLE V. PARASITISM IN THE SAMPLE EXAMINED IN JULY 1938

Height mm.	No. of snails	No. parasitized Observed	No. parasitized Expected*	Difference
5·0	20	11	10	1
5·3	24	20	13	7
5·6	30	22	18	4
5·9	42	31	26	5
6·2	22	20	15	5
6·5	32	26	23	3
6·8	37	32	28	4
7·1	20	15	16	−1

* From the mean incidence of parasitized specimens in the material as a whole.

Incidence and Effect of Metacercaria A

This metacercaria, which was always found encysted, occurred in 3·3% of the snails averaging 2·0 mm. in height, and in 87% of those of 8·3 mm. or more. The degree of infection with this species also increased in the larger snails (Fig. 10). Infections were classed as light, medium or heavy, when 1–6, 6–25, or more than 25 cysts, respectively, were found in any one snail. These criteria were slightly modified in the largest and smallest sizes. Sometimes the whole spire was packed with cysts.

The males were more heavily parasitized than the females by this species (Fig. 11), and altogether 60·35% of the former and 52·7% of the latter were infected. Snails containing no other larval trematodes account for 47·6 and 28·7% of the total, respectively, and the remaining 12·75% of the males and 24·0% of the females were infected with Cercaria B (Fig. 9, and p. 61). There is a thus a reversal of the ratio of infection in the sexes, which may be due to some physiological disturbance caused by Cercaria B. This trematode usually causes sterility, and the sterile males are apparently less attractive to metacercariae about to encyst.

The mere presence of these metacercariae does not affect the seasonal development of the gonads; both males and females containing cysts produced ripe sex cells at the same time as uninfected snails. From September to April, inclusive, that is, during the breeding season, the percentages of snails with unripe, ripe and spent gonads were, respectively, 12·3, 60·0, and 27·7 in 65 snails with heavy infections, and 13·8, 77·8 and 8·2 in 152 with medium infections. The higher percentage of spent snails in those with heavy infections may include a number that were sterile, and it is quite probable that the normal development of the gonads may be inhibited through the resulting pressure when the spire is packed with cysts.

Incidence and Effect of Cercaria B

Cercaria B belongs to the *Ubiquita* Group. It was less common in the smaller size groups than Metacercaria A (Fig. 9). Infections with this species were nearly always heavy, and large numbers of both sporocysts and active cercariae were usually present. Infections with this species occurred in 14·65% males, and 29·7% females, the relative difference between the sexes being much greater than in infections with Metacercaria A when total

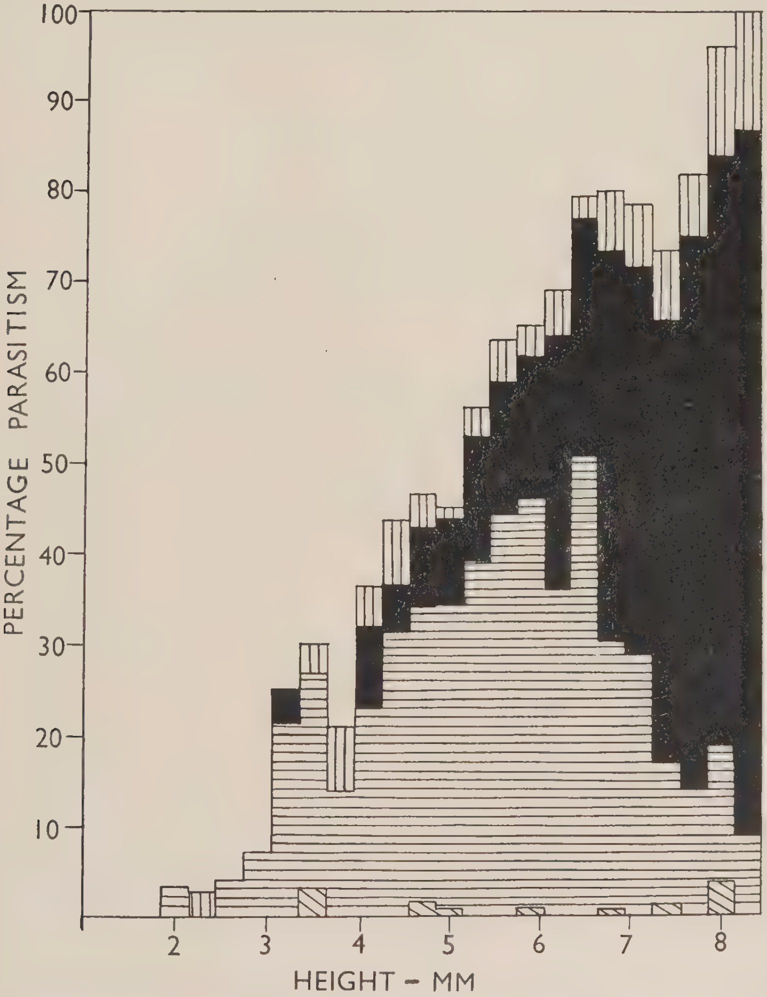

Fig. 9. Percentage parasitism in 0·3 mm. size groups (both sexes). The species of trematode larvae are indicated as follows: Metacercaria A, horizontal shading; Cercaria B, vertical shading; A plus B, solid blocking, C, diagonal shading.

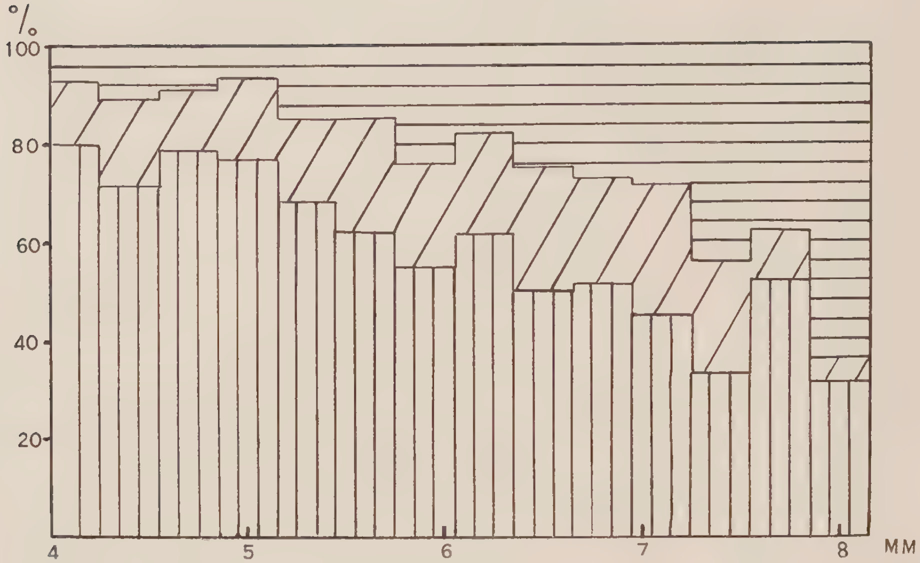

Fig. 10. Grades of infection with Metacercaria A in 0·3 mm. size groups. Vertical shading = light, diagonal = medium, and horizontal = heavy infections.

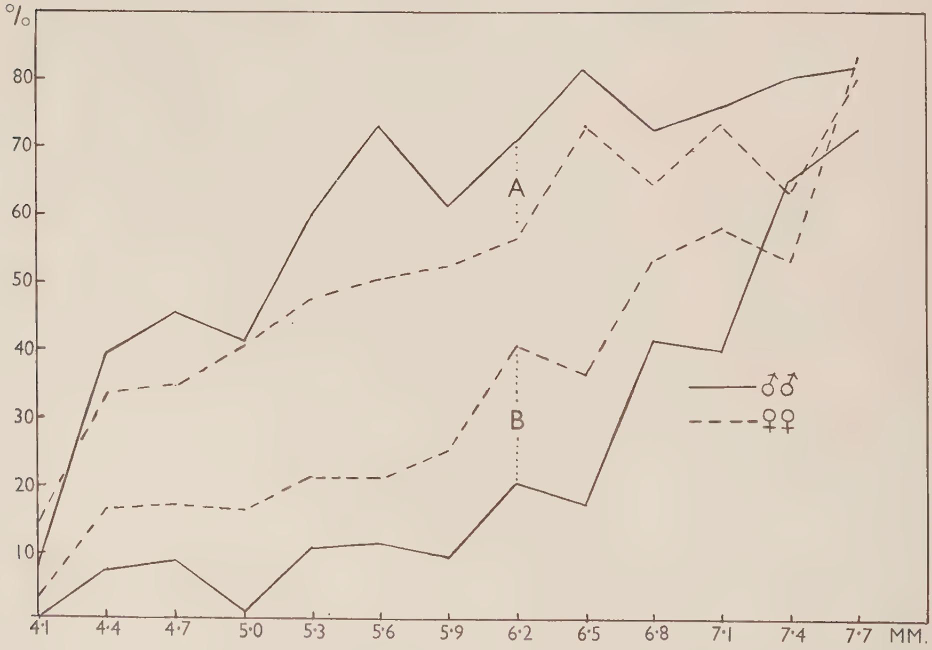

Fig. 11. Differential infections of males and females with Metacercaria A and Cercaria B.

infections are considered. The presence of Metacercaria A together with Cercaria B scarcely alters the ratio of the infections with the latter species in male and female snails, lowering it by only 1·8 % in the males and 5·7 % in the females.

During the breeding season sex cells were found in only 1 out of 86 males, and 8 out of 292 females infected with Cercaria B, so that without doubt this species usually causes sterility.

The differences in the infections of the sexes with Metacercaria A and Cercaria B are of particular interest, as male Gastropoda are usually more heavily infected than females (Pelseneer, 1928). Rothschild (1938*b*), who discusses the question in some detail, found that this was so in *Peringia ulvae*, which is a heavily infected species. Total infections have generally been considered, and it is possible that, when the incidence of separate species of trematodes has been worked out, differences such as occur in *Littorina neritoides* will prove to be common.

Incidence and Effect of Cercaria C

Cercaria C, which is closely allied to *Cercaria emasculans*, was comparatively rare and was found in only 2·23 and 2·81 % respectively of the male and female snails examined. During the breeding season 3 out of 10 infected males and 8 out of 15 infected females were sterile; these numbers are obviously too small to give any indication of the effect of this trematode on the host. Cercariae and sporocysts were both abundant in the infected snails.

Double Infections. The total number of snails infected with Cercaria C was 78; 13 of these contained Cercaria C alone, 45 Cercaria C together with Metacercaria A, and 20 Cercaria C together with Cercaria B. These last 20 infected snails were all sterile. I have followed Cort *et al.* (1937) in considering these last-mentioned as true double infections and distinct from examples in which encysted metacercariae occur with active larval forms. In these double infections both types of cercaria, B and C, appeared equally active. As the total percentage infections with these trematodes were 23·3 and 2·5, respectively, the expected mean number of double infections in the 3019 snails examined is 17·5 ± 4·2. As 20 double infections were observed there does not seem to be any mutual antagonism between these trematodes, such as Sewell (1922) has postulated for some species.

Cercaria D

This larval trematode, which belongs to the *Yenchingensis* Group (Rothschild, 1938*a*) was found only once. Active cercariae and rediae, together with a fair number of cysts of Metacercaria A, were present in a female snail 5·8 mm. high, collected at the beginning of May 1938. No eggs or oocytes were present.

Sex Reversal

There is no evidence that sex reversal occurs in *Littorina neritoides*. The penis diminishes greatly in size in males infected with Cercaria B, but this reduction is no greater than the reduction in non-breeding males during the summer months, and is certainly not comparable with that found by Rothschild (1938*b*) in *Peringia ulvae*.

Incidence of Trematode Parasites in other Localities

Metacercaria A was found in snails from Kelsey Head (north Cornwall), Drake's Island, and in two localities in County Antrim, Sheep Island and the Giants' Causeway. Cercaria B was found in those from Kelsey Head and Sheep Island; and Cercaria C in those from the Giants' Causeway. Notes on the samples from these places and from others where no parasites were found are summarized in Table VI.

TABLE VI

Locality	Date	No. of snails examined	Range of heights mm.	No. of snails infected with cercariae A	B	C
Crantock	30. xii. 35	7	4·3–6·0	0	0	0
,,	14. iv. 36	90	4·6–6·0	0	0	0
Kelsey Head	26. xii. 37	25	4·9–7·2	8	5	0
Rum Bay	10. xii. 35	26	1·9–5·4	0	0	0
Drake's Island	4–20. ii. 36	55	2·8–7·2	11	0	0
Tinside, Plymouth	23. i. 36	100	2·5–6·0	0	0	0
Sheep Island	26. vi. 39	60	5·5–6·9	11	2	0
Giants' Causeway	28. vi. 39	56	4·3–6·6	6	0	2
Gwbert (Cardigan)	8. viii. 39	19	3·4–5·7	0	0	0

All the snails in which parasites occurred were collected from rocks rising steeply from the sea with a high degree of exposure.

Presence of possible Alternate Hosts

Kelsey Head is opposite a small island, the Chick, frequented by gulls, shags and cormorants. Sheep Island is a breeding ground for a large colony of puffins, and numerous razorbills, guillemots and gulls. The rocks there were so bespattered with droppings that a high degree of parasitism might have been expected, but trematodes were quite scarce in the small sample of snails that was examined.

The Breakwater is a resting place for large numbers of several species of gulls (*Larus* spp.) and, less regularly, of flocks of curlew (*Numenius a. arquata*). A flock of about 30 purple sandpiper (*Calidris m. maritima*) overwinters there, and oyster catchers (*Haematopus ostralegus*) occur regularly except in the nesting season. The only invertebrate that is comparable in abundance with *Littorina neritoides* is a small harpacticid copepod which is very plentiful in the pools along the top.

CONCLUSIONS

The observations described show that in the Plymouth area *Littorina neritoides* only spawns when it is submerged. The population is concentrated about the level of extreme high water springs, and the snails do not migrate downwards, so that except in severe storms spawning can only take place at the fortnightly spring tides. Furthermore, spawning is confined to the winter months when tidal levels are likely to be raised by stormy conditions.

The choice of the very dry habitat usually regarded as typical seems to be of doubtful value for a species with pelagic egg capsules. It is probable that it is primarily the need for shelter from the force of the waves that brings about the steady migration to the dry rocks at the top of the zone, and that except for the requirements of spawning the presence or absence of water is only of secondary importance for the adults.

Evidence is given which suggests that the larvae are only able to settle in the barnacle zone on exposed rock faces devoid of fucoids. The snails have very slight powers of adhesion, so that the degree of exposure that eliminates fucoids and is thus favourable to the metamorphosing larvae is unfavourable to the adults. The presence of a fortnightly spawning rhythm seems to be a secondary adaptation which is, perhaps, very deeply impressed. Thus, on the Plymouth Breakwater, where many of the snails find the maximum amount of shelter by living permanently submerged, spawning appears to be as rhythmical as when the snails are living in an excessively dry habitat.

SUMMARY

An account is given of the distribution, life history and trematode parasites of *Littorina neritoides* on the Plymouth Breakwater; a few observations from other localities are included.

The smaller snails are most abundant on the exposed southern slopes of the Breakwater. Many of the larger ones live more or less permanently in water in small cylindrical pits on the top of the Breakwater. This habitat is apparently very similar to that described by Fischer-Piette (1932) as occupied permanently by this species at Cap Martin; Lebour (1935) has sometimes found it in water in other places in the Plymouth area.

The conditions necessary for metamorphosis of the larvae, the need of the adults for shelter from the force of the waves, and the requirements for spawning appear to be of more importance in determining the choice of habitat than the negatively geotactic and varying phototactic responses found in this species by Fränkel (1927).

The breeding season lasts from September to April. The males are ripe about two months before most of the females. Experimental evidence is given which makes it most improbable that there is any downward migration for spawning. From the examination of plankton samples it appears that there is a fortnightly spawning rhythm coincident with high tides, and that even the snails living in water discharge their eggs only at these periods.

The distribution of the size groups is discussed. The proportion of males decreases significantly in the larger size groups and it is probable that there is a difference in the growth-rate of the sexes.

Three species of cercariae and one metacercaria parasitize the snails. The total percentage parasitism increases with the size of the snail, and no kind of immunity appears to develop. Males were more heavily infected with the cysts of the metacercaria than were females, but the latter were more heavily infected with the sporocysts and cercariae of a species that caused sterility.

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APPENDIX

Notes by Dr H. B. Moore on the occurrence of Littorina neritoides *in some localities in Scotland*

9. vi. 36. Head of Loch Fyne. Very weedy shores. No *Chthamalus stellatus* or *Littorina neritoides*. Little *Balanus balanoides*.

11. vi. 36. Caolas Scalpay, Skye. Weedy shore. *Balanus balanoides* abundant in patches. No *Chthamalus stellatus* or *Littorina neritoides*.

18. vi. 36. Port na Cullaidh, Elgol, Skye. Western aspect, sheltered by Soay to the west, but otherwise 180 degrees exposure. All three species abundant on a reef of rock. Very few algae above the *Laminaria* zone.

15. vi. 36. Sgoir Beag, Vaternish, Skye. Aspect, west-north-west. Wave-beaten shore, few algae above the *Laminaria* zone. *Chthamalus stellatus* occupying a zone 50 to 100 cm. above *Balanus balanoides*. *Littorina neritoides* very abundant.

22. vi. 36. Bay of Stoer, Sutherland. Aspect, south west. Wave-beaten rocks. Little weed above the *Laminaria* zone. *Balanus balanoides* abundant. Small zone of *Chthamalus stellatus*. *Littorina neritoides* present.

24. vi. 36. Geodha chobhair, Sutherland. Aspect, west-north-west. Very wave-beaten coast. No algae except in pools and in the *Laminaria* zone. *Balanus balanoides* abundant. Few *Chthamalus stellatus*. *Littorina neritoides* present.

4. vii. 36. Bass Rock. Wave-exposed shore. Very little weed. *Balanus balanoides* abundant. No *Chthamalus stellatus*. *Littorina neritoides* very rare and scattered.

THE EFFECT OF TREMATODE PARASITES ON THE GROWTH OF *LITTORINA NERITOIDES* (L.)

By Miriam Rothschild

(Text-figs. 1–3)

INTRODUCTION

Observations and measurements made on the Gastropod mollusc *Peringia ulvae* (Pennant) 1777 in the wild, suggested that infection with trematode larvae (parthenitae and cercariae) produced gigantism in the host. Experiments in the laboratory (A. & M. Rothschild, 1939) showed that infected snails not only attained ultimately greater size, but also grew faster than uninfected specimens.

Unfortunately, it is difficult if not impossible to compare the growth-rate of an uninfected population with an infected one in nature, as very slight changes in environment (Rothschild, 1938) produce relatively enormous differences in size. Thus, for example, *P. ulvae* collected a few feet apart, on an apparently uniform and identical stretch of mud-flat give different growth curves. In the laboratory simple experiments have confirmed this. Young *P. ulvae* of under 2 mm. in length, were kept for three weeks in water contaminated with gull faeces and subsequently changed to clean water. Their mean size at the end of six months was approximately half that of the controls which were kept in clean water throughout the experiment.

It was hoped to obtain a suitable standard of comparison for gauging the effect of trematodes on the growth of *P. ulvae* by discovering some common parasite which itself exerted no such effect, and to study its distribution throughout a population. A trematode using this snail as second intermediate host only, seemed the most obvious object of study. Unfortunately all the plentiful metacercariae found in *P. ulvae*, either use it as both first and second intermediate host, or else encyst on the outside of the shell. At first it was thought the latter might be utilized, as the chance of finding these cysts on the shell increased proportionately with the age of the snail. Experiments, however, showed that the cysts tend to be knocked off the shells without leaving traces of their presence, and it was therefore found impossible to utilize these species.

During Dr Averil Lysaght's study of *Littorina neritoides* (1941, this volume, pp. 41–67) it was discovered that this mollusc was commonly infected with a Ubiquita cercaria using the snail as first intermediate host, and a metacercaria using it as second intermediate host only. Dr Lysaght very kindly sent me her manuscript and invited me to publish a comment on this portion of her paper, thus giving me the opportunity of making the desired comparison. I would like to express my appreciation and gratitude for this exceedingly generous suggestion.

I would further like to thank Mr G. M. Spooner for his invaluable assistance, Miss Nora Sproston for her help in dissecting experimental gulls and chickens, and Mr W. Searle for collecting *L. neritoides* although exposed to shrapnel and gunfire.

Trematode Infections of *LITTORINA NERITOIDES*

Dr Lysaght records four species of trematode larvae from this mollusc, but both Cercaria C, a species allied to *Cercaria emasculans* Pelseneer, and Cercaria D, a Notocotylid cercaria, occur too infrequently for inclusion in this study.

The commonest species met with, Metacercaria A,* using *Littorina neritoides* as second intermediate host only, is of uncertain systematic position, possibly allied to *Cercaria tuberculata* Fil. It forms a thick double-walled spherical cyst, slightly flattened dorsoventrally, and is found encapsuled in the liver and gonads of the host. Dr Lysaght states that it occurred in 3·3 % of the snails with a mean height of 2 mm., and in 87 % of those measuring 8·3 mm. The number of cysts per snail increases in the large specimens, but their presence does not affect the seasonal development of the gonad. This metacercaria was found in snails infected with Cercaria B as well as in uninfected specimens. In fact, there appeared to be definitely more of these double combinations than should be expected by chance distribution alone.

The second common species, Cercaria B, is a typical Ubiquita cercaria using *Littorina neritoides* as first intermediate host only. Dr Lysaght found the percentage of infection very high for a marine mollusc, 14·65 for males and 29·7 for females. As already stated many of these infections also harboured Metacercaria A and there were a certain small number of true double infections when Cercaria B was found in conjunction with Cercaria C which also uses this snail as first intermediate host. The presence of these cercariae caused sterility in both sexes.

The distribution of both Metacercaria A and Cercaria B in the population of *L. neritoides* is shown in Fig. 1, where the percentage of infection is plotted against size. Dr Lysaght draws attention to the fact that Cercaria B is less common in the smaller size groups than Metacercaria A. In addition to this it will be seen that the two curves are different. The one shown for Cercaria B is fundamentally the same as that demonstrated for *Peringia ulvae* infected with larval trematodes (see Fig. 2). Both display the comparatively low rate of infection in the small size groups and the rapid increase in infection rate in the large size groups, resulting in a steep curve upwards from the 6 mm.

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* *Littorina neritoides* was examined from the Breakwater, Plymouth, the same locality from which Dr Lysaght obtained her material. Metacercaria A was fed in large numbers to laboratory-reared gulls and chicks with negative results.

group (5 mm. for *P. ulvae*) to a maximum of 91 % (100 % for *P. ulvae*) in the 8 mm. group (7 mm. for *P. ulvae*).

The curve for Metacercaria A, on the other hand, does not slope steeply upwards, but increases more or less uniformly to 87 %. In view of the observations made in the wild on *P. ulvae* and the evidence obtained in the laboratory with this species, it is suspected that the dissimilarity in the two curves is due to growth stimulation by Cercaria B. Moreover, assuming that infection occurs at random and increases proportionately with the age of the snail, a far more probable computed growth curve is obtained if it is based on the curve shown for percentage infection of Metacercaria A (see below).

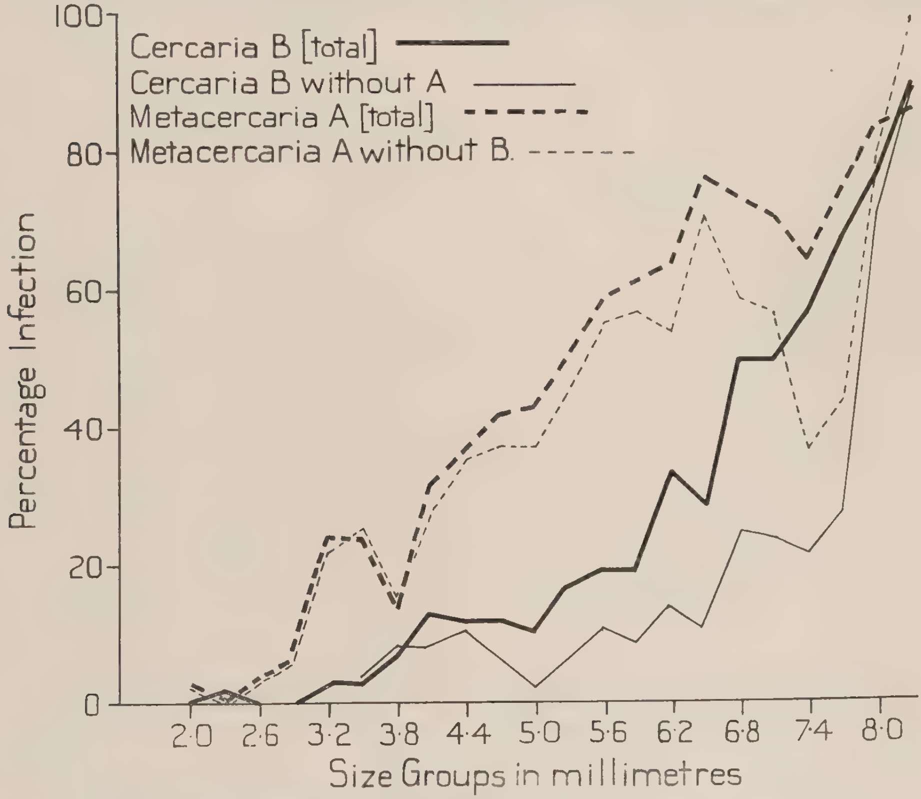

Fig. 1. Percentage infection of population of *L. neritoides*, from data in Lysaght (1941, Fig. 9).

The depression in the upper portion of the curve for Metacercaria A is an anomaly which at present cannot be explained, and it is perhaps idle to speculate as to its possible cause. It does not, however, invalidate the arguments put forward above, as this deviation would, if anything, obscure the contrast obtained for the two curves.

These figures were submitted to G. M. Spooner for criticism and he has very kindly analysed them and made the following comments.

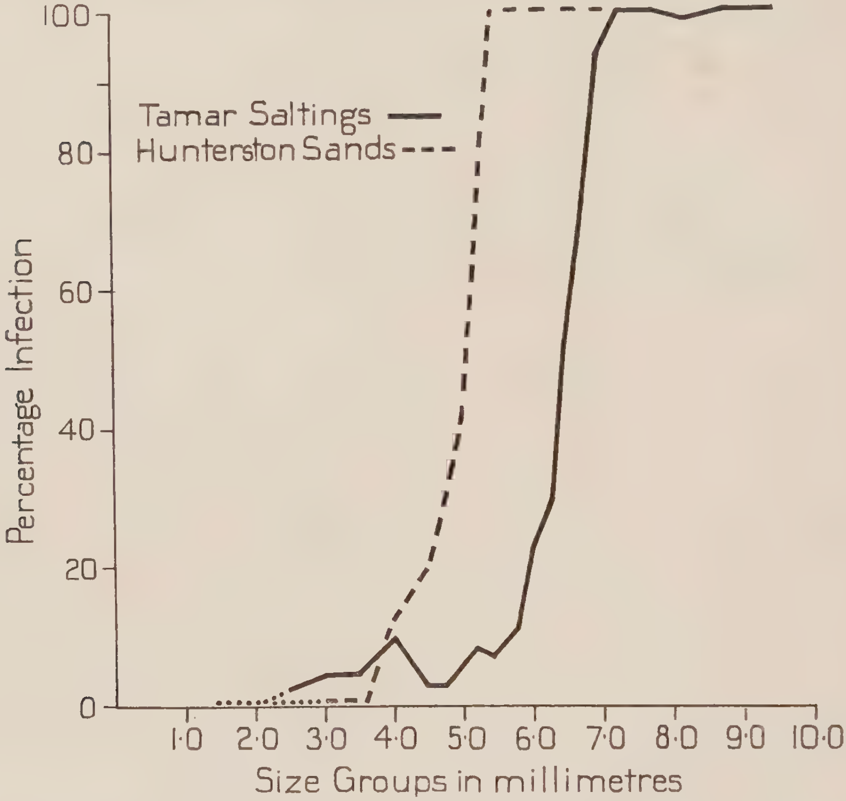

Fig. 2. Percentage infection of 2000 *Peringia ulvae* from Egypt Saltings, River Tamar, and Hunterston* Sands, Firth of Clyde.

Interpretation of the Infection Curves

The curves in Fig. 1 show how percentage infection increases with the size of the snail, or, if the diagram is inverted, how the percentage of the population remaining uninfected decreases with size. Size is a function of age, though the exact relation remains at present unknown. A decrease in freedom from infection as size increases is clearly to be expected, since the longer the snail lives the smaller is the probability that it has avoided contact with the trematode. But does this effect completely account for the shape assumed by both curves?

The method of approaching this question which gives the most instructive results appears to be the following. It is permissible to assume that the average rate of infection of each trematode remains constant, or at least is independent of the size of the snail. In this event the percentage infection of a population plotted against time gives an ordinary exponential curve:

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* Written erroneously as "Hunterdon" in Rothschild (1938).

$$i = 100\,(1-\epsilon^{-at}),$$

in which i is the percentage infected, t is the number of time units, and a is a constant expressing the rate of infection. The formula for the percentage remaining uninfected (u) is of simpler form. From the above,

$$\frac{100-i}{100}=\epsilon^{-at}, \text{ hence } \frac{u}{100}=\epsilon^{-at}.$$

This is a simple logarithmic relation:

$$\log_\epsilon u - \log_\epsilon 100 = -at,$$

hence $$\log u - 2 = -0{\cdot}4343at, \quad\text{or}\quad 2-\log u = kt$$

(in which $k = 0{\cdot}4343a$). Thus the logarithm of the percentage infected falls off uniformly with time.

It follows at once that $\log u$ can be used to represent t, and so provide a time scale. If, therefore, size is plotted against $\log u$ (instead of, as in Fig. 1, $1-u$ against size), the snail's growth curve is obtained. This curve, exemplified in Fig. 3, represents the only growth curve from which the particular percentage-infection curve obtained could have arisen. It is, of course, a limitation that the time units in which it is expressed are of unknown value: or rather, the time scale can only be expressed in fractions of a life span, which is of unknown duration. This defect, however, does not prevent a significant test being made—namely, whether the growth curves derived from the percentage-infection curves of the two trematodes are compatible.

The data for Metacercaria A may be considered first. The size intervals are plotted against $\log u$, in descending scale of the latter, and curve *A* drawn through the points (Fig. 3). If $\log u$ is replaced by $2-\log u$, an ascending time scale is given ranging from 0 to 0·88, this range representing a nearly complete life span of the snail (actually it is the interval between the age at which infection is first possible, presumably during the first few months of life, and the age at which this particular population reaches an average size of 8·3 mm.). If t is time expressed in years, then the scale $2-\log u$ is in units of kt, k being a constant depending on the annual rate of infection.

Curve *B* is the curve similarly derived from the data of Cercaria B. Its time scale, $2-\log u$, is in units of $k_1 t$. k_1 is not identical with k, since the infection rates of the two trematodes are independent, and indeed, as inspection of Fig. 1 suggests, evidently different, that for Cercaria B being the smaller. For the purpose of comparison the two curves should be superimposed so that the ratio between the units of the two time scales is that of k_1 to k (i.e. of a_1 to a, or the ratio of the two infection rates). If no other factor affects the original percentage-infection curves than the most straightforward conditions which have been assumed, the two growth curves should then be coincident. In Fig. 3 the curves are actually superimposed so that the two agree closely over a long stretch of the proximal part of their range, the ratio k/k_1 emerging as 4·0. But it will be seen that towards their distal ends they diverge sharply, and the two complete curves have a very different appearance. Curve *B* indicates a life span of almost five times that given by curve *A*. If, on the other hand, they had been superimposed so that their distal ends coincided, giving both an identical range, then the proximal ends would be conspicuously divergent, curve *B* rising much more sharply than curve *A*.

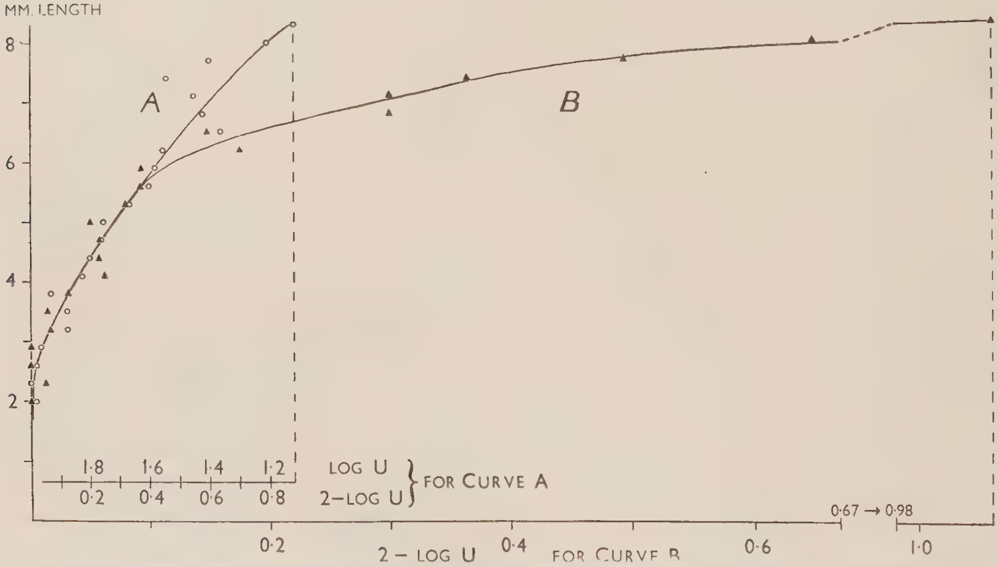

Fig. 3. Theoretical growth-curves of the *Littorina* deduced from the percentage-infection curves of the two trematodes. *A*, curve from the data of Metacercaria A (small circle plots); *B*, curve from the data of Cercaria B (black triangle plots). *u*, percentage of specimens of snail free from infection. For explanation see text.

The growth curves derived from the data of the two trematode infections are thus grossly dissimilar. Both cannot represent the true growth curve of the snail in the population investigated. One at least of the curves must be false; hence one of the original percentage-infection curves requires some special interpretation. This is the first conclusion reached. It is possible to proceed further and enquire which of the two curves is anomalous.

If it is a question of choosing between curves *A* and *B* for the true growth curve of the *Littorina*, there seems little doubt that *A* gives a truer picture than *B*. From what is known of growth-rates in molluscs, and from the available evidence for *L. neritoides* itself, there is little hesitation in assessing curve *B* as extremely improbable. It is, for example, scarcely credible that the snail takes $7\frac{1}{2}$ times as long to grow from 6 to $8\frac{1}{2}$ mm. as it takes to reach

6 mm. On the other hand, there is nothing remarkable about curve *A*. Its form is one which might well have been expected, if it is remembered that the habitat involved is peculiarly favourable to growth. It shows quite an appreciable, and more or less steady, decrease in growth-rate with age, a property which would have been more apparent in the graph itself, had not the time scale been abnormally contracted for the sake of securing space for curve *B*. The second conclusion, then, is that it is the curves derived from the data of Cercaria B which are anomalous: there is clearly an undue concentration of snails infected with this cercaria among the larger size-groups.

This conclusion holds whatever may be the cause of the anomaly. One of the few possible causes (see Discussion) is that the parasites affect the growth of infected snails. On this interpretation the present evidence affirms that Cercaria B stimulates growth, rather than that Metacercaria A inhibits it. This is in complete accordance with evidence from other sources.

It should be noted that the above computations are based on the figures for the percentage *total* infection by each of the two trematodes. In Fig. 1 another pair of curves is plotted, representing the percentage infection, by each trematode, in that section of the population not infected by the other. It happens that the course of this pair of curves consistently lags behind that of the other pair, since combined infections are appreciably in excess of chance expectations, and single infections correspondingly lower. This second pair of curves becomes increasingly unreliable towards the upper end, as the numbers on which the percentages are based are rapidly reduced in the larger size groups, and this loss of accuracy renders these curves much less satisfactory for the treatment given to the first pair, even if the information they contain is considered more important. However, the results obtained from them, as far as they go, are in entire agreement with those given by the total infection data: in fact an even greater contrast between the two trematodes is indicated.

Discussion

In earlier papers the author (Rothschild, 1936, 1938) has briefly discussed the accumulation of specimens infected with trematode larvae (parthenitae and cercariae) among the larger snails—an apparently widespread phenomenon.* This may be due to several causes other than growth stimulation by the parasite:

(1) The young snails may be unattractive to the miracidia and consequently immune to their attack: the infection rate will thus increase with age.

(2) Infections may be lethal to young snails which are consequently killed off.

(3) The growth-rate of the snails may be so greatly slowed down after attaining a certain size, that the time factor alone accounts for the greatly increased percentage of infection in the larger size groups.

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* It is of interest to note that in localities where Cercaria B and C were not found in *L. neritoides*, the largest snails (with one exception) did not exceed 6 mm. in height.

In considering all these possibilities the paucity of direct evidence in the wild is a serious handicap. For reasons which will be indicated later experimental evidence, particularly of a negative character, can only be accepted with the greatest caution. It is possible that all these factors may play some part in bringing about the accumulation of infected specimens in the largest size groups, even if the author's view is correct and the direct effects on the growth-rate of the host by the parasite, is the principal explanation of the distribution curves shown in Figs. 1 and 2.

The *immunity of immature snails* to infection was first postulated by Kemp & Gravely (1919) and Manson-Bahr & Fairley (1920). It would appear that these authors tended to overlook the fact that the time factor must account for a much smaller number of young snails becoming infected. When the rate of infection is low, giving a total percentage of about 1 % as in *L. littorea* (L.) for example, one must expect to examine several thousand baby snails before encountering a single infection. Up to quite recent times no examination of large numbers of the smallest size groups was carried out, and a general impression was gained that these were immune.

There is quite a large body of experimental evidence which goes to show that miracidia may exert intra- as well as interspecific selection, at any rate in the laboratory. This interesting fact can account for otherwise inexplicable phenomena encountered in the life histories of trematodes and their hosts. Although young snails have sometimes proved immune, in other cases the immunity appears to be conferred on old snails, thus for example: "The older the snail (*Segmentina nitidellus* Mtd. and *Planorbis schmackeri* Cless) the less liable it is to infection....Old snails hold over from the previous season, but these are scarcely ever successfully invaded by miracidia" (Barlow, 1925). Again "Old snails (*Pomatiopsis lapidaria* Say) could not be infected with the miracidia of *Paragonimus* sp., but an infection of almost 100 % was obtained in young snails of 1 mm. in length..." (Ameel, 1934). Krull (1931, 1934*a*) also found that full-grown snails (*Gyraulus parvus*) could not be infected, but snails of one or two days old were susceptible. There is consequently little experimental evidence to support this theory.

Mortality of Young Infected Snails. The same can be said for the second supposition put forward. As a whole there appears to be a remarkable adjustment between host and parasite, and infected snails undoubtedly survive for long periods. In the laboratory *Littorina littorea* has lived five years (Meyerhof & Rothschild, 1940) and *Peringia ulvae* four years, giving off cercariae throughout that period. However, the literature contains many scattered statements which indicate that as a whole, infected snails of all ages are less resistant than uninfected ones (Thomas, 1883; Manson-Bahr & Fairley, 1920; Sewell, 1922; Wesenberg-Lund, 1934; MacHattie, 1936; Porter, 1938; etc.). A sudden change in environment is more apt to kill off parasitized specimens. There is also little doubt that certain species of trematodes such as the Echinostomes (Rankin, 1939; Rothschild, 1938) have a more lethal effect than others. On the other hand, Mattes (1936) found that *Zebrina detrina* survived longer when infected than uninfected. It has often been shown that young experimentally infected snails survive to produce cercariae. Krull (1933, 1934*b*), however, found that there was a high mortality of young infected snails and in the case of *Fossaria modicella* Say infected with *Cotylophoron cotylophorum* (Fisch.) and *Fasciola hepatica* L. all died before cercariae producing began. In connexion with the former species he writes: "The snails made little or no growth after having been infected and...it seems reasonable to assume that the amount of food necessary to mature an infection is either not sufficient or does not increase fast enough in a developing snail when infection takes place while the snail is very young."

It must be sufficiently stressed that evidence obtained by laboratory experiments is liable to prove misleading. Again and again failure has resulted from attempts to infect snails with miracidia in artificial surroundings, as apart from other factors, a very slight change in the environment of the snail may render it unsusceptible. Mattes (1936) found it impossible to infect certain snails in his laboratory, but achieved a 100 % infection of the same specimens in his garden. Similarly in the laboratory he successfully infected 100 % of *Helicella candidula* with *Dicrocoelium lanciatum* S. & H., but only 6 % of *Zebrina detrina*, whereas in nature the infection rates were equal. In the laboratory snails may succumb which under natural conditions would survive. Direct evidence in the wild, both in *Peringia ulvae* and *Littorina neritoides*, proves the specimens measuring less than 2 mm. are infected and that these live at any rate until the infections produce cercariae.

Depression of Growth Curve. When considering the third possibility it must be recalled that Sewell (1922) pointed out that Gastropods showed an accelerated growth-rate in the first months of their life. It is also well known that the growth of these molluscs slows down considerably with increasing age. Both Moore (1937) and Lysaght (1941) have demonstrated this slowing down of growth in *L. littorea* and *L. neritoides* in their third and fourth year—a phenomenon much more marked in this genus than in *Peringia*. However, as shown above, an unusual type of growth curve would have to be postulated for *P. ulvae* and *Littorina neritoides* in order to account for the distribution of trematodes throughout the population, supposing infection occurs purely at random.

The Behaviour and Effects of Metacercariae. The facts concerning Metacercaria A recorded by Dr Lysaght agree on the whole with the known observations made on metacercariae which use molluscs as second intermediate host only. Generally such infections appear to occur at random throughout the population, the number of cysts per snail increasing with the time it is exposed to infection. Some examples, however, are reported which clearly indicate selection on the part of the free-swimming cercariae. Thus certain species show a tendency not to re-enter snails infected with their own sporocysts, though readily entering those infected with other species (Winfield, 1933; Nolf & Cort, 1933). In Metacercaria A it would appear that the free-swimming cercaria is attracted by snails already infected with Cercaria B. A more probable explanation of the high number of these combined* infections is that the sluggish behaviour of infected snails gives the cercariae a better chance to penetrate.

Apart from possible mechanical obstruction caused by the presence of large numbers of cysts, snails appear to be little if at all affected by the presence of metacercariae† using them as second intermediate host only. There is no active destruction of the tissues and sterility does not result. Dr Lysaght draws attention to this fact for Metacercaria A. It is the fact that metacercariae apparently cause no serious histopathological changes in the host, which makes them a suitable subject for comparison with primary infections.

Although no further conclusions can be reached with regard to the actual cause‡ of the large size of snails serving as first intermediate host for trematodes, such a comparison tends to heighten the belief that these parasites induce growth.

SUMMARY

If the number of infections with (*a*) trematode parthenitae and cercariae using *Littorina neritoides* as first intermediate host only, and (*b*) encysted metacercariae using *L. neritoides* as second intermediate host only, are plotted against the size of the snails, two different curves result. The first shows a low rate of infection in the small size groups, but a steep upward slope rising to 91 % in the large size groups. The second shows a curve increasing uniformly to 87 % infection.

Possible interpretations are discussed, and it is concluded that the difference is probably due to the fact that primary infections cause accelerated growth in the host.

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* The term "double infection" is reserved for infestations with two primary infections, i.e. two species of cercariae each using a mollusc as first intermediate host. The term "combined infection" is proposed for infections consisting of parthenitae of one species using the snail as first intermediate host and metacercariae of another species using it as second intermediate host.

† This term does not include Tetracotyle which are known to be detrimental to the snail (Van Haitsma, 1930).

‡ The suggestion was originally put forward that castration might account for the increase in size of infected snails. *Littorina littorea*, artificially castrated by treatment with X-rays in doses of 400 r. at 150 kV. and 4 mA., screened with 4 mm. Al., did not grow any faster or any larger than controls.

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OBSERVATIONS ON THE NIGHT TIDAL MIGRANT CRUSTACEA OF KAMES BAY

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INTRODUCTION

During the year 1936 Mr Richard Elmhirst, Director of the Scottish Marine Biological Station at Millport, carried out a series of night tow-nettings across the waters of Kames Bay* at varying times after nightfall, mainly between the hours of 10 p.m. and midnight. The series was taken in order to ascertain the nature and extent of the faunal immigration into the bay from outside waters, and the nocturnal and vertical movement of the fauna which normally lies buried in the sand during the day. The net employed was an ordinary coarse tow-net supplemented on occasions by a finer net; the latter net, however, captured but few macro-Crustacea, and its results will be neglected in the present paper. The method employed was to walk into the water at the edge of the incoming or outflowing tide to a depth of about 2 ft. and to walk across the bay following the edge of the tide trailing the net about 10 ft. posteriorly and at arm's length into the tidal flow, thus avoiding as far as possible disturbances set up in the water by the walking movement. The length of tow was approximately 100 yards. Thus the position of each sample is determined by the time it was taken in relation to the day of the lunar month, and samples were obtained at all levels from high-water mark to low-water mark. I have previously dealt with the species of the amphipod genus *Bathyporeia* which occurred in these samples (Watkin, 1939). In this paper the remainder of the macro-crustacean fauna apart from the mysids is analysed. I am indebted to Mr Elmhirst for handing over these samples to me for analysis, and for his critical comments during the investigation.

An appreciation of the fauna of the samples is amplified by a knowledge of the fauna which normally lives in the intertidal sand of the bay. This enables the distinction to be drawn between those species which perform simple vertical migrations from the sand into the intertidal waters and those which are carried in by the tide into the intertidal area from the various habitats which lie beyond the low-water mark of spring tides. For this purpose a series of sand samples for faunal analysis was taken during the last week of March and the first week of April 1939. It is realized that a period of over two years separates the time when the tow-net samples were taken from that when the sand samples were obtained. It is known, however, that no major differences in the fauna occurred in the meantime, although differences in the density of a particular species may have occurred. The general conclusions are not invalidated by this time difference.

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* A map and a brief description of Kames Bay has been given in a previous paper (Watkin, 1939, p. 470).

The method of faunal analysis of the sand was to fix stations, at 5-yard intervals, from the high-water mark to the low-water mark of spring tides along a sampling line previously used in the investigations of Elmhirst (1931) and Stephen (1928 and subsequent dates) into the sand fauna of the bay. At each station a block of sand of 1 sq. ft. surface area and of a depth of 6 in. was removed and sieved through a brass wire sieve of 30 meshes to the linear inch. The detailed analysis of this series of samples will form the subject of a separate paper. For the purposes of this paper reference will be made only to the species which also occur in the night tow-net samples.

A comparison of the species which occur in the night tow-net samples with those of the sand samples shows that the night migrant fauna falls into two distinct groups.

(*a*) Those species which normally lie buried in the intertidal sand during the day and perform vertical movements only, retaining their zonation when swimming in the tidal waters. These are the amphipods *Bathyporeia pilosa* Lindström, *B. pelagica* (Bate), *B. elegans* Watkin and *B. guilliamsoniana* (Bate), *Pontocrates norvegicus* Boeck, *P. arenarius* (Bate) and *Urothoë brevicornis* Bate; the isopod *Eurydice pulchra* Leach; and the cumaceans *Pseudocuma cercaria* (van Beneden) and *Cumopsis goodsiri* (van Beneden). The above species represent the dominant forms that occur in the sand, apart from *Haustorius arenarius* (Slabber) which did not appear in the night tow-net samples. Apart from these an occasional specimen of *Nototropis swammerdami* (M.-Edw.), *Megaluropus agilis* Hoek, *Lamprops fasciata* Sars, *Iphinoë trispinosa* (Goodsir) and *Crangon vulgaris* Linn. also occurred in the sand samples.

(*b*) Those species that do not occur in the intertidal sand, but are carried in with the inflowing tide from various habitats beyond low-water mark and thus perform a horizontal, in addition to a vertical, migration. These habitats may be classified as (1) the sand habitats which extend outwards from low-water mark to the deeper waters of the Firth of Clyde, and (2) the algal habitats. The latter harbour during the day species which cling to clumps of seaweed on the sea floor beyond the low-water mark of spring tides, and may also include the algal dwellers of the rock shore, which, until further evidence is available, may be expected to contribute to the night migrant population. The main species in the second group are the amphipods *Gammarus locusta* (Linn.), *Nototropis swammerdami* (M.-Edw.), *Calliopius rathkei* (Zaddach), *Dexamine thea* Boeck, and *Apherusa* spp.; the isopod *Idotea viridis* (Slabber) and the mysid *Schistomysis spiritus* Norman. Other species which occur only rarely in the samples are *Marinogammarus* spp., *Megaluropus agilis* Hoek, *Stenothoë monoculoides* (Montagu), *Podoceros falcatus* (Montagu), *Metaphoxus fultoni* (T. Scott), *Periculodes longimanus* (Bate & Westw.), *Erichthonius braziliensis* (Dana), *Siphonoecetes colleti* Boeck, *Phtisca marina* Slabber, *Hyale* sp., *Iphinoë trispinosa* (Goodsir), *Lamprops fasciata* Sars, *Diastylis rathkei* (Kröyer) and *Bodotria scorpioides* (Montagu).

The rare appearance of such algal dwellers as *Stenothoë*, *Podoceros* and *Phtisca* is probably due to the drift of bits of algae, and of the tube dwellers such as *Erichthonius* and *Siphonoecetes* to storm action; they cannot at the moment be regarded as night migrants. The sand dwellers, such as *Megaluropus*, *Periculodes*, *Metaphoxus* and the cumaceans, may perform extensive vertical migrations over their respective habitats.

THE FAUNA OF THE SAND

The analysis of the crustacean population of the sand samples is given in Table I.

TABLE I. THE ZONATION OF THE MAIN SPECIES OF CRUSTACEA

Station no.	*Eurydice pulchra*	*Urothoë brevicornis*	*Pontocrates norvegicus*	*Cumopsis goodsiri*	Station no.	*Eurydice pulchra*	*Urothoë brevicornis*	*Pontocrates norvegicus*	*Cumopsis goodsiri*	*Pseudocuma cercaria*	*Pontocrates arenarius*
13	1	—	—	—	29	2	24	128	4	—	—
15	35	—	—	—	30	6	33	141	4	—	—
16	8	—	—	—	31	—	22	188	9	—	—
17	13	4	—	—	32	5	40	221	2	—	—
18	5	3	—	—	33	1	26	201	14	4	—
19	8	1	3	—	34	1	17	105	8	—	—
20	3	13	11	—	35	1	18	112	4	2	—
21	10	22	29	—	36	—	30	84	5	7	—
22	16	12	30	—	37	1	25	36	5	—	3
23	3	24	71	—	38	—	25	59	2	5	3
24	4	14	52	—	39	—	31	36	3	2	2
25	2	11	78	—	40	—	42	24	4	17	37
26	3	20	86	—	41	—	19	29	2	22	17
27	3	26	96	—	42	—	6	15	2	34	43
28	7	32	90	7							

High-water neap tide occurs at St. 14, approximate mid-tide at St. 21, low-water neaps at St. 39.

An analysis of the night tow-net samples for the four species of the genus *Bathyporeia* has been given (Watkin, 1939). Consequently the detailed figures of the distribution of these species in the sand samples are omitted. Further, only those species which occur in the night tow-net samples and whose distribution extends over several stations are included. The following species also occurred: *Lamprops fasciata* St. 35, one; *Iphinoë trispinosa* St. 42, two; *Nototropis swammerdami* St. 42, two; *Megaluropus agilis* St. 42, one; and *Crangon vulgaris* St. 24, one.

The first five stations from the high-water mark of spring tides contained no macrofauna. *Bathyporeia pilosa* appeared at St. 6 with a centre of maximum abundance at Sts. 12 and 13, gradually disappearing to St. 22. *Haustorius arenarius* ranges over Sts. 10–14 and spasmodically to the low-water mark of spring tides. The high-water mark of neap tide occurs at St. 14 (recorded by Mr Elmhirst on May 12 1939, calm sea with a slight north-westerly breeze). *Eurydice pulchra* extends from the upper neap-tide level with a maximum at St. 15 and occurs continuously in varying abundance to St. 37 with a definite tendency to be more abundant in the upper half of the neap-tide range. *Urothoë brevicornis* first appears at St. 17 and continues to the low-water mark of spring tides with a tendency to be more abundant in the lower half of the neap-tide range. The low-water mark of neap tide occurs at St. 39. *Pontocrates norvegicus* extends from St. 19 in gradually increasing abundance to St. 32, to be followed by a gradual decrease to low-water mark. *Bathyporeia pelagica* first appears at St. 24, increases to a maximum over Sts. 31–35 and disappears after St. 40. Thus this species and *Pontocrates norvegicus* have a similar centre of maximum abundance in the lower half of the neap-tide range. The former is distinctly an intertidal species in this bay, but the latter may extend beyond the low-water mark of spring tides. *Cumopsis goodsiri* is never abundant, but is represented at every station from 28 to low-water mark, and thus does not occur above mean sea-level. *Pseudocuma cercaria* does not appear until St. 33 and does not become abundant until St. 40, i.e. below the low-water mark of neap tides. *Bathyporeia elegans* first appears at St. 35 and extends to the low-water mark of spring tides with a centre of abundance near low-water mark. *B. guilliamsoniana* also appears in the last six samples, but is known to be definitely more abundant beyond low-water mark. *Pontocrates arenarius* appears in the last seven samples with a suggestion that its centre of abundance is near low-water mark.

The Fauna of the Night Tow-net Samples

Table II gives the dates on which the samples were obtained, the time the sample was taken, the time of afternoon high tide, the age of the moon in days and the number of individuals of the most abundant species per sample. Table III shows the data for the most important species from the point of view of zonation and also summarizes the results of a detailed analysis of the maturity of each individual.

Pontocrates norvegicus and P. arenarius.

P. norvegicus was present in thirty out of the forty-eight samples and *P. arenarius* in nine, with six samples showing a mixture of the two species. However, in all the six samples containing both species one of the species is

TABLE II. NUMBER OF INDIVIDUALS OF THE MOST ABUNDANT SPECIES IN EACH SAMPLE

Day and month 1936	Age of moon in days	G.M.T. p.m.	Time of high water p.m.	*Pontocrates norvegicus*	*Pontocrates arenarius*	*Nototropis swammerdami*	*Calliopius rathkei*	*Gammarus locusta*	*Idotea viridis*	*Eurydice pulchra*	*Pseudocuma cercaria*	*Cumopsis goodsiri*
7. i.	14	10.00	11.44	—	—	—	—	2	—	3	—	—
15. i.	22	7.30	4.16	117	—	2	—	6	—	—	—	—
21. i.	28	6.45	10.32	28	—	1	—	18	—	2	—	—
12. ii.	20	8.30	3.7	4	28	10	5	25	1	—	76	—
13. ii.	21	7.00	3.37	110	—	1	—	1	—	—	—	—
27. ii.	6	7.40	3.32	11	—	244	2	1656	31	—	—	—
4. iii.	12	11.00	10.38	—	—	—	—	1	—	1	—	—
19. iii.	27	9.00	9.34	—	—	—	—	—	—	—	—	—
24. iii.	2	4.30	1.00	—	—	—	—	—	—	—	—	—
29. iv.	9	10.15	6.53	—	—	—	—	33	—	2	—	—
30. iv.	10	10.15	8.10	125	—	—	—	36	1	4	—	—
1. v.	11	10.15	9.13	10	—	—	—	28	2	1	—	—
2. v.	12	10.15	10.2	2	—	—	—	42	—	7	—	—
3. v.	13	10.15	10.44	2	—	—	1	5	—	9	—	—
9. v.	19	10.15	1.55	91	—	1	1	1	2	12	1	—
14. v.	24	10.15	5.55	—	—	—	—	17	—	17	—	—
15. v.	25	10.15	7.9	59	—	3	—	115	55	2	1	1
16. v.	26	10.15	8.17	42	—	1	—	35	27	12	—	—
17. v.	27	10.15	9.19	13	—	—	—	23	4	15	—	—
21. v.	2	10.15	0.22	19	—	—	—	—	—	22	—	—
1. vi.	13	10.15	10.0	—	—	—	—	—	—	1	—	—
10. vi.	22	11.00	3.49	120	—	—	—	11	1	2	—	1
12. vi.	24	10.30	5.32	6	167	31	—	41	5	1	72	1
16. vi.	28	10.30	9.52	—	—	—	—	—	—	—	—	—
23. vi.	5	1.00*	3.12	—	—	—	—	5	—	1	—	—
26. vi.	8	11.00	5.12	2	219	11	—	42	—	—	90	—
27. vi.	9	1.00*	5.58	479	1	5	—	31	1	1	1	29
2. vii.	14	11.00	10.53	—	—	—	—	2	1	—	—	—
14. vii.	26	11.00	8.23	44	—	—	—	128	2	1	—	—
21. vii.	4	11.00	2.9	786	7	1	—	13	—	—	15	23
31. vii.	14	11.00	10.30	—	—	—	—	6	2	24	—	—
12. viii.	26	11.00	8.11	235	—	2	1	102	3	3	1	9
21. viii.	5	11.00	2.41	21	103	101	1	121	—	2	80	—
31. viii.	15	11.00	11.35	—	5	—	—	6	—	7	2	—
8. ix.	23	9.45	5.11	9	43	7	—	46	2	1	227	—
15. ix.	1	10.00	0.6	150	—	1	—	1	1	18	2	3
24. ix.	10	10.00	5.28	18	—	3	1	2	—	1	1	1
30. ix.	16	10.00	11.50	84	—	—	—	—	—	3	2	7
5. x.	21	10.30	3.9	—	18	99	8	9	—	1	259	—
20. x.	6	10.30	2.41	70	—	3	—	3	1	13	—	4
28. x.	14	10.30	10.38	—	—	—	—	1	—	2	—	—
7. xi.	24	8.30	7.0	—	—	—	—	7	—	3	—	—
12. xi.	29	10.30	11.24	109	—	3	—	503	31	4	3	—
25. xi.	12	8.30	9.14	—	—	—	—	—	—	—	—	—
11. xii.	28	11.45	10.58	—	—	—	—	15	—	2	—	—
14. xii.	2	8.45	0.25	8	—	—	2	10	3	12	—	1
14. xii.	2	11.00	0.25	2	—	—	—	2	1	16	—	—
22. xii.	10	7.30	6.19	—	—	—	—	—	—	2	—	—
Total				2776	591	530	22	3151	177	230	833	80

* 1.00 a.m.

represented by a few individuals only. The samples are reasonably pure to one species. It is to be expected that samples taken towards low-water mark would contain both species, since the analysis of the sand fauna shows that they overlap from St. 37 seawards. *P. arenarius* is present in considerable numbers only in those samples taken between L.W.N. and L.W.S. (Table III), e.g. 12. vi. 36 and 26. vi. 36. The sample of 31. viii. 36 is aberrant, it was taken near the high water of a spring tide and was the only occasion when this species was taken outside its zone, as shown by the sand samples. Similarly, the samples containing *P. norvegicus* in abundance were those taken in the middle and lower third of the neap-tide range, e.g. 21. vii. 36, and 27. vi. 36. The samples taken at high water contain none or very few. Thus both these species retain their sand zonation when swimming in the intertidal waters. A comparison with those samples containing *Bathyporeia* (Watkin, 1939) shows that *P. arenarius* is present in the same samples as those containing *B. guilliamsoniana* and *B. elegans* and only partly with those containing *B. pelagica*, whilst *P. norvegicus* overlaps in the main with *B. pelagica* and partly with *B. pilosa*.

TABLE III. SUMMARY OF THE ZONATION AND MATURITY OF SOME SPECIES

	Number of samples	*Pontocrates norvegicus*	*Pontocrates arenarius*	*Nototropis swammerdami*	*Calliopius rathkei*	*Urothoë brevicornis*	*Megaluropus agilis*	*Gammarus locusta*	*Idotea viridis*	*Eurydice pulchra*	*Pseudocuma cercaria*	*Cumopsis goodsiri*
					ZONATION							
H.W.S. to H.W.N.	4	2	—	—	—	1	—	45	1	10	—	—
Upper third neap-tide range	15	254	5	3	1	1	—	640	40	75	5	1
Middle third neap-tide range	12	966	—	10	2	3	—	446	91	95	7	20
Lower third neap-tide range	12	1472	154	363	6	8	—	1900	38	35	324	54
L.W.N. to L.W.S.	5	82	432	154	13	1	72	120	7	15	497	5
					ANALYSIS OF MATURITY							
Adult males	—	455	22	31	6	6	20	27	Not examined	Not examined	122	12
Females with eggs	—	545	50	54	2	—	28	11			337	17
Adult females without eggs	—	182	56	184	13	6	16	5			202	7
Immature males	—	422	33				8	3108			72	23
Immature females	—	458	109								86	21
Immature—sexes indistinguishable	—	714	321	261	1	2	—				14	—
Total	48	2776	591	530	22	14	72	3151	177	230	833	80

Bearing in mind that some of the sample are taken outside its tidal range, the presence of *P. norvegicus* in thirty out of the forty-eight samples suggests that it is migratory throughout the year. Its density, however, on various nights differs considerably.

A detailed examination of the individuals showed that in *P. norvegicus* all stages of maturity are present at all times of the year. Egg-bearing females and adult males were recorded for each month and the spasmodic appearance of numbers of young forms suggests that broods are produced at definite times. The data are not full enough to draw definite conclusions on the number of broods produced, but they suggest that one brood per month is released. Similar remarks also apply to *P. arenarius*, but no samples, apart from that of 12. ii. 36, were taken within their zone during the first six months of the year.

The totals (Table III) show that the migrant population of *P. norvegicus* consists of 19·6% egg-bearing females, 16·4% adult males, 6·5% non-egg-bearing females, 31·7% immature forms of both sexes and 25·7% young forms. Thus 42·5% are adults. The preponderance of adult males is not nearly so marked as in *Bathyporeia*, where 42% were adult males. The percentage of adult females is, however, somewhat higher, 26·1% as compared with 15%. Comparing these figures with those obtained for the sand population, taken in late March and early April before the production of the new season's broods has reached a high level, when 61% were adults with 5% adult males, it is seen that there is a tendency for adult males to be more abundant in the tow-net samples. The individual samples are somewhat variable in their population, but where the number of individuals in a sample is high the population consists of a mixture of forms in all stages of maturity.

The migrant population of *P. arenarius* shows a smaller percentage of adult males and immature males than *P. norvegicus*, but otherwise retains the same general features.

The data are not sufficient to draw definite conclusions in regard to lunar periodicity. Those given in Table II show irregularity during the first half of the lunar month but become more regular during the last 10 days of the month, with a suggestion that the species becomes more abundant in the periods immediately preceeding the new and full moon.

Fage (1933) records both species in his night tow-nettings "autour d'un foyer lumineux" at Concarneau and Port-Vendres; the numbers are small with adult males predominating.

Urothoë brevicornis (Tables I and III).

It has been noted that this species occurs abundantly in all the sand samples from St. 17 to low-water mark. Its occurrence in the night tow-net samples is, however, very spasmodic. A total of fourteen individuals is recorded from nine samples; they are spread over the whole range in which this species occurs in the sand samples, showing, as in the sand samples, a tendency to be more abundant in the lower third of the neap-tide range. The fourteen individuals comprise six adult males, one adult female, five immature females and two young forms. Fage (1933) records other species of this genus in his night tow-nettings "autour d'un foyer lumineux", particularly at Concarneau. All the specimens were males. Russell (1925) records the genus in samples taken up to 50 m. depth in the waters of the English Channel on a moonlight night in July, and notes that it differs from other amphipods in appearing in the surface waters at 9 p.m. Tattersall (1913) records *U. marina* and *U. elegans* as night migrants in the Clare island survey. Thus there is considerable evidence to show that species of this genus are migratory at night; but none of the records indicate the presence of egg-bearing females.

The allied form *Haustorius arenarius* is recorded in the sand samples but not in the tow-net samples. Dennell (1933) remarks that "*Haustorius* apparently may forsake the sand...to swim freely" and under laboratory conditions it was observed to do so between the hours of 11 p.m. and 6 a.m. It is to be expected that a species so modified for a swimming habit should have occurred in the samples, but I can find no reference to its occurrence as a night migrant.

Gammarus locusta (Tables II and III).

This species inhabits "water of some fathoms depth" (Elmhirst, 1932) and is usually regarded as living amongst algae. It is thus a migrant into Kames Bay from its known habitat in deeper waters, and is the predominant migratory amphipod from outside waters.

From its presence in forty out of the forty-eight samples it is reasonable to suppose that it is a tidal migrant throughout the year at all phases of the moon. Its abundance is, however, exceedingly variable. Note may be made of the sample of 27. ii. 36 with 1656 individuals and that of 12. xi. 36 with 503 individuals, with many samples containing less than ten. The samples with high numbers do not seem to bear any relationship to the state of the tide at the time they were obtained. They occur in the low-water, mid-tidal and high-water samples. Similarly, they are spread evenly throughout the year. When the data for the whole year are plotted against a lunar month it is seen that there is a distinct tendency for the numbers to increase as the period of new moon approaches and to a less extent towards the period of full moon, if the one exceptional sample of 1656 individuals, taken on the sixth day of the lunar month, is omitted. The numbers fall during the immediate periods of full and new moon.

A striking feature of the results, which is characteristic of every sample, is the dominance of the immature stages and the progressive reduction in numbers as size increases. Thus of 3151 individuals 1495 (47 %) are under 3 mm. 1276 (41 %) from 3 to 5 mm., 280 (9 %) from 5 to 7 mm., 69 (2 %) from 7 to 9 mm. and 31 (1 %) over 9 mm. Blegvad (1922) in his account of the life history of this species in Danish waters records that males attain full maturity at a size of 10 mm. and some females may attain maturity at 6 mm. Thus this species is characteristically a migrant in its young stages. It should be noted, however, that forty-three, or 1·4 %, of the individuals are adults with the males predominating, twenty-seven of the forty-three. Of the sixteen adult females eleven carried eggs in the brood pouch. The presence of a few adults prompts the suggestion that possibly they are more numerous in the waters over their immediate habitat and that they do not perform such extensive horizontal migrations as the young forms. Further sampling should prove interesting. Fage (1933) records a somewhat similar population at Concarneau, in which the young forms predominate over a few adult males.

Mr Elmhirst is of the opinion that this species comes in when there is much drift algal detritus in the tidal waters.

Marinogammarus spp. occurred in five samples only: No. 8, one; No. 19, one; No. 42, one; No. 46, one; No. 47, four. Of the eight individuals seven were young forms with one egg-bearing female. The data are scanty but the same general features as in *G. locusta* are indicated.

Nototropis swammerdami.

The habitat of this species is amongst the sublittoral algae. Table II shows that it occurred in twenty of the night tow-net samples, which indicates that it is migratory throughout the year; but as with the other species the extent of the migration is very variable. Three of the samples account for 84 % of the total number recorded. The samples in which it occurs were taken at all stages of the tide, but it only occurs in abundance in those taken in the lower third of the neap-tide range and those near low water. Continuous sampling in this region would probably show its occurrence to be more regular. All stages of maturity are represented (Table III), with breeding females present only in the samples with a large number of individuals and forming 10 % of the total. Adult males are relatively scarce, 6 % of the total, with the young forms predominating. Thus this species shows migratory features similar to those of *Gammarus locusta*. Fage (1933) records both *N. guttatus* and *N. swammerdami*, the population consisting mainly of adult males, and Tattersall records *N. vedlomensis* as a night migrant in the Clare Island survey. Other authors also record its pelagic habit.

Calliopius rathkei.

Nine samples scattered over the year contain this species in small numbers. The data are scanty but suggest that it is more numerous during the winter than the summer months, and, as with *Nototropis swammerdami*, it is abundant in the low-water samples. The habitat is amongst sublittoral algae; Sars (1891) records that it occurs in Norwegian waters "in great shoals, swimming actively near the beach, and ascending it according as the tide flows up". Tattersall records it as a night migrant.

Apherusa spp.

This genus is represented by the three species *A. jurinei* (M.-Edw.), ten individuals in seven samples, *A. bispinosa* (Bate), six individuals in three samples, and *A. cirrus* (Bate), one individual, occurring from May to December. It is noteworthy that the individuals are either adults or immature forms and none of the adult females were egg-bearing. Many authors record the pelagic habit of the species in this genus. Thus Russell (1927) records the abundance of *A. ovalipes* and *A. clevei* in the day-time in the waters of the English Channel; they were absent from the surface layers but abundant from 20 m. depth, 10 min. hauls giving 3480, 6480 and 6940 individuals. In dealing with hauls taken at night-time in summer, he further records (1931), that during 1925 (and probably showing the same behaviour in 1926) there was a marked upward movement at night, the species being evenly distributed from the surface downwards. Samples taken throughout the year 1930 and part of 1931 (Russell, 1934) show the species to be abundant from late June to September, reappearing in April 1931. Fage lists *A. bispinosa* at Concarneau with males and females in almost equal numbers and notes that the species is exceptional in that its abundance is not affected by the light employed.

Other amphipods.

Attention may be drawn to *Dexamine thea*, seventeen individuals from six samples, and *Stenothoë monoculoides*, seven individuals from five samples, as adding to the list of algal dwellers which may be included under the species performing a night migration. The data for the sand dwellers *Megaluropus agilis* (Table III) and *Periculodes longimanus*, six individuals from two samples, does not represent the extent of their migration, since Elmhirst (1932) has shown that samples taken at two fathoms depth near the low-water mark of spring tides yield these two species in considerable numbers. The samples were taken on a night in March and again in June and successive sampling at 3 hr. intervals from 6 p.m. to 6 a.m. gave maximum hauls at 9 p.m. and midnight. *Metaphoxus fultoni* was recorded in one sample only, but its occurrence as a night migrant in the waters of the Firth of Clyde is recorded by Patience (1909) in samples taken during July at one fathom from the bottom. Males were more abundant than females. *Podoceros falcatus*, three individuals from three samples, *Erichthonius braziliensis*, two individuals from two samples, *Siphonoecetes colleti*, two individuals, *Phtisca marina*, one individual, *Hyale* sp. must be regarded as chance occurrences due to storm action.

Eurydice pulchra.

This species occurs in thirty-seven of the samples (Table II). The samples indicate its presence throughout the year and at all tide levels, with a tendency, shown also by its distribution in the sand, to be more abundant in the upper and middle portion of the neap-tide range. It is probable that this species is as equally a day migrant as a night migrant, although actual figures are not available to substantiate this statement. Observations carried out on this species in the laboratory show that it obtains food by capturing other living organisms in the plankton, tearing a rent in their skin and disembowelling them by the use of the anterior peraeopods. On the beach it may be observed that they rise out of the sand with the inflowing tide, proceed to feed immediately and sink again into the sand with the retreating tide. The migration into the tidal waters is thus essentially a feeding one. Details of the annual life cycle are not available but casual observations suggest that two broods, a spring and an autumn, are produced.

Idotea viridis.

The occurrence of this species in twenty-one of the samples suggests that it is migratory throughout the year from its habitat in the littoral and sublittoral algae. The numbers in the samples are very variable, four of them accounting for 81% of the total. A notable feature is the dominance of young forms with the numbers rapidly decreasing as size advances. The figures are, under 2 mm. 60, at 3 and 4 mm. 73, at 5 and 6 mm. 32, at 7 and 8 mm. 4, over 8 mm. 8. Thus those under 4 mm. comprise over 75% of the total. Howes (1939) records that in a saline lagoon in Essex this species attains maturity at 5 mm. in the female and 6 mm. in the male, although very few are mature at this size. Assuming that this population attains similar sizes the absence of the adult stages becomes notable. One egg-bearing female was obtained. When the data for the whole year are plotted against a lunar month it is noted that the species occurs throughout the month but with a definite tendency to be more abundant in the period immediately preceeding the new moon. The migrations of this species recall those recorded here for *Gammarus locusta* and a comparison of the sample numbers shows that the maximum number of both species occurs in the same samples. Elmhirst is definitely of the opinion that the occurrence of *I. viridis* is associated with drift weed in the tidal waters. Thus the amount of algal detritus must be considered as a contributory factor in the migration of both species.

Fage (1933) draws a distinction between the pelagic migration of the Cirolanidae on the one hand and the Idoteidae on the other. In the former the egg-bearing females always remain associated with their "substratum" whatever that may be, and the pelagic migrants are either males or "young" forms, whilst in the latter the thigmotropism which associates them with their substratum during the day is obscured at night-time and all stages of maturity are represented in the night migrant population. This conclusion is partially substantiated by the above observations on *Eurydice pulchra* and *I. viridis*.

Cumopsis goodsiri.

Table I shows that this species occurs in the sand samples from St. 28 to low water mark, and, although never abundant, it occurs in every sample. Table II shows that it was present in eleven of the tow-net samples between May and December but only in numbers during late June and July. The samples in which it occurs were taken mainly within its range as shown by the sand samples, its abundance occurring in the lower third of the neap-tide range; but its absence from many samples suggests that it is spasmodic in its migratory habits. Of the eighty individuals obtained seventeen were egg-bearing females and twelve adult males, with immature forms of both sexes predominating and young forms absent.

Pseudocuma cercaria.

Of the forty-eight samples sixteen include this species. It will be recalled that it only occurs in sand samples taken near low water. Similarly, it only occurs in numbers in those night tow-net samples taken either at the low-water level of spring tides or in the lower third of the neap-tide range. An occasional specimen occurs in the mid-tidal samples and exceptionally in the upper third of the neap-tide range. It must be regarded mainly as a tidal immigrant from the sand below low-water mark, although the centre of its abundance needs further investigation. It differs from other tidal immigrants in being restricted to low water. Many authors have commented upon the relationship between cumaceans and moonlight. The present data when arranged on a lunar month basis shows two definite peaks of density; the first over the 5th–8th day and the second over the 20th–23rd days, thus suggesting an association with lunar illumination of a certain intensity.

Elmhirst (1932), in a series of samples taken every three hours from 6 p.m. to 6 a.m. on a night in March and again in June, shows that this species is abundant in the surface waters at low tide; but at high tide, when the low-water level is covered by 2 fathoms of water, it is abundant near the sea floor and practically absent from the surface. His data further show that very few are carried in with the tide to high-water mark. The above observations substantiate in part the observations of Elmhirst, but in this investigation no depth samples were taken.

In Table III the sex and degree of maturity of the individuals are shown. The adult males were mainly of the "short antennae" type (Foxon, 1936, p. 388). Very few "fully adult" males were found. The data show that egg-bearing females are present from February to October and that they represent 40 % of the total catch. If the adult females without eggs are included, the percentage rises to 65 %, adult males accounting for 9 % only. Thus adults predominate, with immature males and females constituting the remainder of the catches. Young forms are practically absent. Foxon, giving counts made on previous collections in this area, records males as more abundant than females and Fage makes the same observation. Further work on more extensive collections is necessary before definite statements can be made.

Other cumaceans.

Two of the low-water samples contain *Iphinoë trispinosa*, which occurs in the sand from low-water mark to some depth. *Diastylis rathkei* was recorded once, *Lamprops fasciata* once, and *Bodotria scorpioides* was found in three samples. Russell (1931) records the vertical movement of the last-named species at night-time.

Crangon vulgaris.

Young forms of this species from 4 to 10 mm. in length occurred in eight samples from June onwards. The numbers were small and a total of only twenty individuals was recorded.

Attention may finally be drawn to occasional species other than the Crustacea. Young Pleuronectids less than 12 mm. in length were present in five of the May samples, and other young fishes in some samples from May onwards. Young Nereids of 3–5 mm. were present in the June and July samples and *Scolelepis* adults in four samples. The use of a coarse tow-net avoided most of the usual smaller plankton organisms, but occasional megalopa and zoea larvae, calanoid and harpacticid copepoda and medusae were found.

Of the forty-eight samples thirty-nine contained Mysids, mainly *Schistomysis spiritus*, in varying numbers and varying degrees of maturity, many of the samples containing numerous young forms. It is hoped that these will be analysed and reported on in due course.

Discussion

As far as I am aware this series of samples, collected by Mr Elmhirst for the study of the night migrant macrofauna of the intertidal waters throughout a whole year, is the first of the kind which has been made. The main purpose of the work is to show that this field is a fruitful one for further investigation. It is not proposed to discuss in detail the possible factors which underlie the migration of the species either individually or as a whole, but rather to indicate the problems requiring further investigation and to show how this series of samples demonstrates the positions at which the collection of further samples should be made. The samples were taken, in all cases, at the edge of inflowing or outflowing tide. Thus the position of each sample is determined by the position of the tide when the sample was taken, and they have served to show the general outline of the problems involved. The main point that emerges, apart from the fact that the major part of the night migrant population consists of Crustacea, is that the population falls into two well-marked groups: (*a*) that which normally lives in the intertidal sand of the bay and becomes migrant at night-time, and (*b*) that which is carried in to the bay by the inflowing tide from other habitats. Thus, as an auxiliary study, it is necessary to know the fauna of the intertidal soil and also that of the adjacent deeper water habitats which may be (1) sand or mud habitats, or (2) algal habitats, or both. This investigation shows that both habitats contribute to the migrant population. To complete the analysis of the migrant fauna it is necessary to obtain samples of the microfauna, which in addition to the usual plankton organisms will consist of such forms as Harpacticids (Elmhirst, 1935), and also to obtain a knowledge of the fishes which both as adults and in their young stages may migrate inshore at night-time in varying numbers at the different seasons of the year (Elmhirst, 1935; Marshall *et al.* 1939). By such studies it will eventually be possible to build up a picture of the extensive activities which occur in intertidal waters at night-time.

The data given in this paper and previously for *Bathyporeia* show that the sand population retains its zonation when migrant in the intertidal waters, but that species which are migrant from outside habitats are distributed over the whole tidal range and show no zonation. Thus, *Pontocrates norvegicus* and *P. arenarius* and the four species of *Bathyporeia* only occur in those samples taken over their immediate habitat, whilst *Gammarus locusta* occurs in practically every sample. The further investigation of the intertidal sand species involves three lines of sampling: (*a*) a series taken above the high-water mark of neap tides which will include as the main species *Bathyporeia pilosa*, (*b*) a series taken just below the mean sea level, i.e. a mid-tidal sample, which is the centre of abundance of *Pontocrates norvegicus* and *Bathyporeia pelagica*, and (*c*) a series at the low-water mark of spring tides to include *Bathyporeia elegans*, *Pontocrates arenarius*, *Pseudocuma cercaria* and *Cumopsis goodsiri* as the dominant species. These three series of samples would also supply much information about the migrants from the deeper water habitats, but they would need supplementing by samples taken over their respective habitats to show whether the population differs at various points along the migratory path. *Gammarus locusta* is a suitable species in this respect. The sampling in deeper waters must include depth samples as well as surface samples; *Pseudocuma*, for instance, is known to remain associated with the water immediately over its habitat and not to rise in numbers to the surface.

Though the data here presented furnish evidence that the behaviour of the species changes at different times of the year, the apparent association of many species with varying lunar light intensities needs as continuous a series of samples as possible. Much further evidence is also required in regard to the time when the species rise, to show if it bears any relationship to the time of the rise of the moon and if it differs on moonlight or moonless nights. This factor may, however, be of considerably less significance than that of the degree of calmness or roughness of the tidal waters. It is probable that there is a close relationship between the calmness of the tidal waters and the degree of emergence of the species from their habitats. In view of all the factors involved, the collection of a perfect series of samples becomes almost an impossibility.

The results for the various species show that their behaviour does not follow any generalized form; but a broad distinction may be drawn, in the amphipods, between the intertidal sand-dwelling forms and the immigrant species. The migrant population in the former, typified by *Pontocrates* and *Bathyporeia*, shows all stages of maturity, whilst in the latter, typified by *Gammarus* and *Nototropis*, there is a predominance of young forms with the adults taking but little part in the migration. In the intertidal sand-dwelling forms adults tend to predominate over the young, with the adult males showing a stronger migratory tendency than the females. The reasons for this difference in behaviour can only be assumed. It may be imagined that the behaviour in both instances serves as a means of dispersal but with a difference in the age of the dispersal phase. It may be that the sand-living forms migrate to form pairs, while this may be possible amongst algal dwellers without the necessity of migration. A more detailed study of the relationship of the species to their habitat is needed, and of their reproductive habits. The latter are known for *G. locusta* from the work of Blegvad, and it is known that many sand-dwelling species feed whilst buried in the sand, and do not migrate for the purpose of obtaining food. In *Eurydice*, however, which is a day migrant as well as a night migrant, the movement is essentially a feeding one and the absence of egg-bearing females indicates that its main purpose is not that of reproduction.

The behaviour of the various species in regard to the lunar cycle shows that *Pontocrates*, *Bathyporeia* and *Gammarus* are similar; this suggests that a factor other than feeding and reproduction may be causative, and the difference in the time of appearance in the lunar cycle between the species of these genera and *Pseudocuma* suggests that the same factor may affect different species in different ways.

SUMMARY

The faunal analysis of forty-eight samples taken with a coarse tow-net across the intertidal waters of Kames Bay during 1936 shows that the night migrant crustacean population falls into two well-marked groups: (*a*) those species which live in the intertidal sand and perform simple vertical migrations at night-time into the intertidal waters, and (*b*) those which are carried in by the tide into the intertidal area from various habitats which lie beyond the low-water mark of spring tides. In the former group the species of the genera *Bathyporeia* and *Pontocrates* are dominant and they retain their zonation as migrants, the population consisting of individuals in all stages of maturity. This is in sharp contrast to the latter group, typified by *Gammarus locusta* and *Idotea viridis*, which occur over the whole tidal range with a population in which the young immature stages are dominant. The Haustoriids *Urothoë brevicornis* and *Haustorius arenarius* occur as inhabitants of the sand, the former appearing occasionally in the tow-net samples and the latter absent from them. The Cirolanid *Eurydice pulchra* performs both day and night migration for the purposes of feeding.

Discussion of the results indicates the lines along which further investigations may be carried out.

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THE PRESERVATION OF FISHING NETS, TRAWL TWINES AND FIBRE ROPES FOR USE IN SEA WATER

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INTRODUCTION

Several papers of this series have already appeared; the last (1936) dealt with nets and its fellow, in collaboration with Prof. J. Purser, with ropes. The best of the methods already tested have now been pitted against new methods or modifications. In particular, preservation by means of cutch, fixed by various processes, received attention.

When considering the best preservative to use, regard must be paid to the thickness of the threads of the net or the diameter of the twines and ropes. With a fine thread, adherence to the relatively large surface may be more important than penetration; whereas with a rope penetration is first and foremost necessary, coupled of course with a low solubility in sea water. Adherence may be improved and leaching away reduced by a subsequent treatment, with tar for example.

The samples were treated in the Laboratory and taken to the sea water basin at Pier Cellars, on the Cornish side of the Sound beyond Plymouth breakwater. This small basin was nearly emptied each tide. In the 1936–7 tests the samples were always totally immersed, being suspended from ropes slung across the basin. In the later tests the ropes were so placed that the samples were out of the water for 3–4 hr. each tide. Some tests were interrupted by the war scare in the autumn of 1938. The samples were replaced in December. All remaining exposures were, however, interrupted by the war in the autumn of 1939.

In order to avoid the use of heavy testing machines a lighter rope than before was used in these tests; all materials were, for uniformity, supplied by one firm, the Gourock Ropework Co., Ltd., as follows:

"Gourock" six thread three strand manila rope, breaking load 700 lb.

Six thread three strand "Yacht" manila rope, breaking load 500 lb., approximately 0·6 in. in circumference.

"Gourock special" manila trawl twine, 150s (namely 150 yards to 1 lb. weight), 160 lb. breaking load.

Cotton netting, 32s/12 ply, 100 mesh deep, 26 rows to the yard. The cotton yarn count is the number of hanks which weigh 1 lb., each hank being 840 yards in length; 32s is therefore 26,880 yards per lb., so with 12 ply the length is 2240 yards per lb.

The strength shown for the new samples is the average value as given by the maker. For samples under 100 lb. tensile strength a spring balance was used; this dial registered the maximum pull. For samples under 200 lb. a dial spring balance was used also, but the needle of this flew back at break, so the figure was hardly reliable to within 10 lb. The ropes tested were so attached, by loops, that only the length under test received any strain. All breaks were clear of the attachments. For samples which did not break in any portion at 200 lb., tension was applied by means of lead weights. Results shown are usually the average of two or three breaks, save with the lead. In some cases, however, one or more portions of sample exceeded the maximum tensile test that could conveniently be applied at the time. Two figures such as 89, 93 and a third over 100 are shown in the tables as an average of >94.

PRESERVATIVES AND THEIR APPLICATION

Coal distillation products

Crude gas-works benzene, used as a solvent for copper soaps; this blackens iron vessels because it contains sulphur compounds.

Motor benzene, a purer solvent, Anglo-American Oil Co.

Creosote oil, purchased locally.

Coal tar from local gas-works; a very thick tar, becomes moderately dry in one month.

Coal tar, "Corroid tar", specially prepared for nets by Messrs Hardman, Hull; a less viscous tar, becomes moderately dry in a fortnight.

"Coalite" low-temperature distillation products

Crude phenol, b.p. 180–230° C., a dark mobile liquid.

A neutral oil, b.p. 170–230° C. ("oil A"). Does not darken much on standing; has been extracted with alkali and with acid; kindly presented by the Director, Chemical Research Laboratory, Teddington, as was also "oil B".

"Oil B", b.p. 225–250° C. at 14 mm. pressure, extracted like oil A.

Coalite neutral oil, b.p. 100–245° C.; sp. gr. 0·971; alkali extracted, darkens on standing. Presented by Coalite Works, Gawber, Yorkshire, as were also other samples.

Coalite heavy neutral oil, b.p. 220–370° C.; sp. gr. 0·975; alkali extracted.

Coalite middle oil, b.p. 180–220° C., with 47% crude tar acids; sp. gr. 0·966.

Coalite heavy oil, b.p. 230–280° C., with 39 % crude tar acids; sp. gr. 1·018.

Coalite low temperature tar, dries moderately in about 3 weeks, rather quicker than the more viscous local tar; all drying rates are approximate and vary with the conditions.

Wood distillation products

Not used in these tests, used formerly, see this *Journal*, 1936, Vol. xx, p. 628.

Petroleum products

White spirit is a distillate between 140–200° C.; it is of low flash point (Shell Co.).

Kerosene or paraffin oil, distils at 150–300° C.; it has a higher flash point, being prepared for domestic use (Shell Co.).

Petrol, as for motor cars (Anglo-American Oil Co.).

Cutch varieties

Cutch from the Bakau and Kenya Extract Co., probably a mangrove extract, but the company's representatives in Glasgow refused all information.

"Forestal brand" cutch, from the heart wood of the South American Quebracho tree, *Quebrachia schinopsis lorentzii.*

"Elephant brand" extract, from the bark of the South African mimosa or black wattle, *Acacia decurrens* var. *mollissima.* These are completely soluble in hot water. For analyses, see Atkins, 1936, p. 629. The samples and analyses were kindly supplied by the Forestal Land, Timber and Railways Co.

Copper compounds

Copper sulphate, crystals, commercial quality. For Olie's (1918) method use a 1 % solution in water and add just enough ammonia solution to redissolve the precipitate; this requires about 44 volumes of ammonia solution (sp. gr. 0·88) for 1000 volumes of copper solution; the resultant liquid is a deep blue; it is used after cutch. Soak 10–15 min., not longer; the solution is nearly decolorized by the net.

Mixed copper soap, containing stearate with oleate, palmitate and laurate, made by Messrs Lever Bros. and sold either ready for use or in a concentrated emulsion. For use with nets it is prepared to contain about 20 % of creosote or of thick mineral oil. We are indebted to Lever Bros. for a number of samples.

Copper oleate, a dark green greasy solid, contains about 2 % of added creosote as supplied by Messrs Wm. Bailey and Sons, Wolverhampton, who also kindly provided samples of resinate and naphthenate.

Copper resinate, a light green dry powder, clean to handle; not very soluble in petrol, soluble in benzol, solvent naphtha and in "oil A" (neutral low-temperature tar distillate).

Copper naphthenate, a dark green greasy solid, sold for use usually in solvent naphtha or various petroleum oils. We have received samples from Messrs Bailey, the Brent Manufacturing Co. in various solvents, and have also obtained it from Scott, Bader and Co. whose source appears to be the Brent Co. It is on the market as "Cuprinol" of various grades. As a result of tests done here a certain proportion of coal tar is now incorporated with the quality sold for nets. It was first manufactured in Denmark many years ago, by Messrs Cuprinol.

A new grade of Cuprinol, specially prepared to give waterproofed fishing lines in the rivers of British Columbia was also tested. This dries with a gloss on the outside of the tin but is not brittle.

Miscellaneous preserving agents

Two organic mercurial preparations, kindly supplied through the "Pest Control Research Committee" and Imperial Chemical Industries, Ltd., were also tried. DS 836 was a miscible oil product, with mercury content 5%. DS 837 was a paraffin oil solution, with mercury content 3·6%. They were used diluted with water, 1 vol. to 100 and 1 vol. to 70 respectively, giving 0·05% Hg.

"Shirlan" was also tried. It is an anti-mildew agent introduced by the British Cotton Industry Research Association for use on sized cloth. It was of course never intended for use in sea water.

A waterproofing process, the Velan treatment, was also tested. The process was applied as directed by the makers, Imperial Chemical Industries, Ltd., who also make Shirlan. Velan PF was used.

"Tectal" is a creosote preservative, prepared by Melanoid, Ltd. of Tipton, one of the Mond group. Through the courtesy of the Belfast Ropework Co., Ltd., we were provided with a sample and analysis, showing its relation to coal-tar oil.

	Coal-tar oil	Tectal
Sp. gr.	1·03	0·99
Flash point	70° C.	90° C.
Boiling range	130–140° C.	200–360° C.
Phenols	10–15%	35%
Viscosity	2·5	1·6

RESULTS OF TESTS

Experiments of 1937–8: *tests with copper soaps* (Table I)

The aim of the tests shown in Table I was to study the relative merits of copper soaps in various solvents and at various dilutions. A few other preservatives were also tried; the most effective of these on nets was a sample believed to have a modified cutch bichromate treatment, No. 28. This did as well as No. 22, copper resinate in tar, and as Lever's copper soap mixture in creosote oil. Very curiously both outlasted No. 9, copper oleate in tar, which is usually the best on nets. Since No. 28 did so well, special attention

Table I

Tensile strengths (mean of three tests) of manila rope, trawl twine and cotton netting immersed in basin of clean sea water, replenished at half-tide but never dry, near Cawsand, outside Plymouth Sound breakwater. Samples immersed about 8 months after treatment. Time in air after removal, before testing, shown in italics. Immersed 27. viii. 37. Tensile strength in lb. Nets tested by hand (G = good).

Sample	Material tested	"Yacht" manila			Twine				Net	
no.	Period of treatment ... (months)	6	12+5	%	3	6	12+5	%	2¼	6
25	Untreated, new or kept in air, dry*	500	—	100	160	—	119*	75	—	—
1	Untreated, immersed	0	—	0	24	0	—	0	0	—
2	Lever's copper soap, ready for use	> 100	153	31	> 95	53	24	15	0	—
3	Do., conc., diluted with oil A, 1–4 vol.	88	76	15	66	9	0	0	0	—
4	Do., conc., diluted with oil A, 1–9 vol.	54	50	10	58	0	—	0	0	—
5	Do., conc., diluted with creosote oil, 1–4 vol.	> 100	149	30	> 85	41	44	27	F.G.	G
6	Do., conc., diluted with creosote oil, 1–9 vol.	> 100	168	34	> 99	32	0	0	0	—
7	"Tectal"	60	39	8	47	0	—	0	0	—
8	Copper oleate 1 lb., "Corroid" tar 1 lb., oil A 1 gal.	> 93	77	15	> 100	21	0	0	0	—
9	Do., 1 lb., "Corroid" tar 1 gal.	> 98	100	20	> 100	20	36	22	V.G.	0
10	Do., 1 lb., "Coalite" tar 2 lb., oil A 1 gal.	> 100	80	16	66	21	0	0	0	—
11	"Cuprinol", special British Columbia grade	> 100	173	35	> 100	**75**	0	0	0	—
12	Do., green for nets, with tar added by makers	> 100	**221**	**44**	67	64	0	0	0	—
13	Do., with equal vol. of petrol	> 100	84	17	> 81	40	0	0	0	—
14	Copper naphthenate 30 % (2·4 % Cu) in naphtha, Brent	> 100	143	29	82	50	45	28	0	—
15	Do., 30 % (2·4 % Cu) in white spirit, Brent	> 100	100	20	> 100	51	0	0	0	—
16	Do., 30 % (2·4 % Cu) in industrial alcohol, Brent	> 100	188	38	> 91	52	55	34	0	—
17	Do., water-resisting mixture in naphtha, Brent	> 100	148	30	> 95	69	**64**	**40**	0	—
18	Do., water-resisting mixture in white spirit, Brent	> 100	139	28	70	58	55	34	0	—
19	Do., preservative mixture, Brent	69	44	9	54	0	—	0	0	—
20	Do., Bailey 10 % (0·8 % Cu), "Corroid" tar 1 lb. per gal., in creosote oil	> 100	100	20	59	0	—	0	0	—
21	Do., Bailey 10 % (0·8 % Cu), "Corroid" tar 1 lb. per gal., in oil A	> 95	83	17	71	0	—	0	0	—
22	Copper resinate 1 lb., "Corroid" tar 1 gal.	90	65	13	53	0	—	0	G	G
23	Do., 1 lb., petrol 1 gal.	30	0	0	26	0	—	0	0	—
24	Do., 1 lb., creosote oil 1 gal.	> 100	100	20	69	0	—	0	F.G.	0
26	Mercurial compound DS 836, dil. to 0·05 % Hg	21	0	0	50	0	—	0	0	—
27	Do., DS 837, dil. to 0·05 % Hg	23	0	0	32	0	—	0	0	—
28	Gourock Rope Co., latest cutch bichromate net treatment	—	—	—	—	—	—	0	V.G.	G

* The three surviving nets had rotted after 7 months.

was paid to cutch bichromate methods in the next series, since heretofore these had given occasional good results but were on the whole erratic. It may be seen that only three nets remained sound for 6 months, and none for longer. In practice, however, retreatments at shorter intervals may lengthen life, as described in Atkins, 1936, Table I. On the other hand seven of the trawl twines were still tolerably strong after a total immersion period of a year and then 5 months in air. These did not include either Nos. 22 or 28, but No. 5 is in, fifth on the list with 27%. A copper naphthenate water-resisting mixture in naphtha, No. 17, was so effective that the twine had 40% of its initial strength after this long immersion. These Brent Co. naphthenate samples provided the first four on this list, but the use of copper naphthenate is conditioned by a suitable solvent, for No. 19, with a low temperature tar distillate failed.

The Cuprinol naphthenate mixtures, one of which headed the trawl twine list after 6 months, all perished with the longer period; Lever's concentrate (which is largely oleate) diluted with creosote oil and copper oleate in tar remained relatively strong.

With the closely twisted "Yacht" manila penetration is more important, and Cuprinol and Brent naphthenates take the first three places, with a more dilute (1 to 9) Lever concentrate a good fourth.

Experiments of 1938–9: *tests with cutch, etc.* (Table II)

Table II is occupied with a remarkable array of failures, nearly all modifications of the cutch treatment. The most successful for nets were cutch bichromate methods, six of which survived the first water immersion test, along with twelve with various copper soap or tar treatments similarly exposed and shown in Table III. As all these perished during air storage while wet, they were repeated in 1939 and four bichromate treatments and eight of those in Table III survived a longer period, 27 weeks in the sea and 14 on a gravel path in the open.

For trawl twines the Gourock sample was best but very poor compared with the methods of Table III; next in Table II comes mangrove cutch with Olie's method or hot bichromate. The same held good for "Yacht" manila rope save that as one sample with Olie's method failed the result was taken as zero. For the Gourock manila Olie's method gave 20% of the initial value after the full 14½ months in water and 6 in air; the same was given by No. 16, a bichromate with sulphur dioxide, and four bichromate dips were somewhat inferior. All, however, are far surpassed by results in Table III.

Experiments of 1939: *further tests on various preservatives* (Table III)

Eight samples of netting survived the more extended 1939 test, which was interrupted by the war. It is probably correct to judge between these survivals by taking into consideration the results with trawl twines which had

TABLE II

Tensile strengths of specimens immersed in sea water basin 4. iv. 38–29. ix. 38. Those sound immersed again 12. xii. 38–9. ix. 39. Tested 20. xii. 39. During air storage, 2½ months in 1938, the nets remaining were left wet in error and all rotted. Life of untreated net 14 weeks first set, 16 weeks second, and 12 weeks for 1939 set. Ropes had 14½ months in water and 6 in air; twines had 7½ and 2½. A and B represent two sets treated similarly.

Sample No.	Material tested … …	Gourock manila rope A lb.	Gourock manila rope B lb.	Gourock manila rope Mean % Dec. 1939	Yacht manila rope A lb.	Yacht manila rope B lb.	Yacht manila rope Mean % Dec. 1939	Trawl twine Mean % Feb. 1939	Cotton net Percentage life
48	Untreated new dry	700	700	100	500	500	100	100	—
1	Untreated immersed	22	26	3	0	0	0	0	100
2	Mangrove cutch	23	24	3	0	0	0	0	110
3	Do., with Olie's method	83	191	20	0	56	0*	17†	115
44	Mimosa cutch	55	112	12	0	0	0	4	105
46	Do., with Olie's method	54	100	11	0	0	0	9	115
45	Quebracho cutch	70	57	9	0	0	0	6	100
47	Do., with Olie's method	84	107	14	0	0	0	12	110
6	As 2, then 5 min. in bichromate 15° C.	91	147	17	0	0	0	14†	> 160‡
7	Do., then ½ min. in bichromate 60° C.	70	120	14	42	36	8	17†	> 160‡
8	Do., then ½ min. in bichromate 40° C.	95	125	16	0	0	0	12†	130‡
9	Do., then ½ min. in bichromate 15° C.	95	150	17	0	0	0	12	> 160‡
10	Do., then 5 min. half-neutralized in bichromate 15° C.	0	92	0	0	0	0	7	115
11	Do., then ½ min. half-neutralized in biochromate 60° C.	27	107	10	0	0	0	11	120
12	Do., then ½ min. half-neutralized in bichromate 40° C.	23	117	10	0	0	0	8	135
13	Do., then ½ min. half-neutralized in bichromate 15° C.	0	120	0	0	0	0	9	130
14	As 8, then fresh water, pass SO$_2$ for 1 min.	36	165	14	0	0	0	8	150
15	As 8, but pass SO$_2$ into bichromate 1 min. before dip	56	110	12	0	0	0	9	145
16	As 9, continue as 14, viz. at 15° C.	150	137	20	26	0	0	13†	> 160
37	Gourock cutch bichromate treatment	—	—	—	52	63	11	22†	> 160
41	Do., new treatment	—	—	—	—	—	—	—	> 160
42	Untreated control for 41	—	—	—	—	—	—	—	110
38	Velan treatment, 2½% room temperature	45	40	6	0	0	0	2	135
39	Do., dried on water radiator and air oven 5 min. at 120° C.	57	72	9	0	0	0	2	105

* When one value is zero the mean is arbitrarily taken as zero.

† All the second set were rotten after the 7½ months in water, save the six marked. These were put back in water; all were rotten 11 weeks later.

‡ On repetition in 1939 these stood 27 weeks and 14 weeks on a gravel path, namely, a life of over 225% in water. Of one set Nos. 37 and 41 were rotten after the air exposure test. The duplicates were then poor.

TABLE III

Tensile strengths of ropes, trawl twine and cotton net immersed in sea water in among the specimens of Table II, and for the same periods, save that second set of trawl twine lasted longer. Ropes had $14\frac{1}{2}$ months in water and 6 months in air, also second set of twines; first set of twines had $7\frac{1}{2}$ months in water and $2\frac{1}{2}$ months in air.

Sample No.	Material tested ...	Gourock manila rope			Yacht manila rope			Trawl twine		Cotton net
		A lb.	B lb.	Mean % Dec. 1939	A lb.	B lb.	Mean % Dec. 1939	A Mean % Feb. 1939	B Mean % Dec. 1939	Percentage life Mean of two
48	Untreated new kept dry	700	700	100	500	500	100	100	100	—
1	Untreated immersed	22	26	3	0	0	0	0	0	100
2	Mangrove cutch	23	24	3	0	0	0	0	0	110
4	Do., then creosote oil cold	190	212	29	57	120	18	36	22	150
5	Do., then "Corroid" tar, cold	250	218	34	82	100	18	36	31	>160*
21	Creosote oil, cold	175	157	24	26	29	5	25	R 20/4†	115
22	"Corroid" tar, cold	212	166	27	27	35	6	29	R 20/4	155
40	Gourock Rope Co. tar, 3 min. at 49° C.	—	—	—	118	120	24	76	R 3/5	>160
43	Do., using Plymouth tar	—	—	—	86	160	25	55	M 11/9	140
23	Copper oleate 1 lb. in "Corroid" tar, cold	>300	>300	> 43	190‡	195‡	38	> 57	M 11/9	>160*
24	As 2, then as 23	>300	>300	> 43	165‡	190‡	35	> 60	35	>160*
25	As 3, then as 23	>300	>300	> 43	150‡	155‡§	30	50	45	>160*
26	Copper naphthenate (Bailey) in tar, like 23	>300	>300	> 43	77	150	23	56	M 3/5	>160
27	As 2, then as 26	>300	>300	> 43	187	160	35	49	35	>160*
28	As 3, then as 26	>300	>300	> 43	193	280‡	47	53	48	>160*
29	Copper resinate in tar like 23	>300	>300	> 43	132	230‡	36	34	M 3/5	>160
30	As 2, then as 29	>300	>300	> 43	270‡	195‡	46	54	41	>160*
31	As 3, then as 29	>300	>300	> 43	190	190‡	38	49	35	>160*
32	"Cuprinol" green for nets	>300	>300	> 43	98	230‡	33	75	24	160
33	"Cuprinol", British Columbia grade	>300	>300	> 43	101	190‡	29	70	21	155
34	As 32, 1 lb. tar extra per gal.	>300	>300	> 43	165	175‡	34	75	25	155
35	As 2, then as 32	>300	>300	> 43	165	150‡	31	47	35	145
36	As 2, then as 33	>300	>300	> 43	210‡	170‡	38	57	48	155
17	Lever's mixture ready for use	>300	>300	> 43	160	125‡	30	36	R 20/12	150
18	As 2, then as 17	300	>300	> 43	174	220‡	39	59	44	>160
19	As 3, then as 17	>290	>300	> 42	97	150‡	25	56	30	150
20	As 2, then as 17‖ in creosote oil, 1–4 vol.	>270	>300	> 41	150	195‡	34	53	36	150

* On repetition in 1939 these stood 27 weeks in Cawsand basin and 14 weeks on a gravel path, namely a life of over 225% in water since untreated control rotted in 12 weeks. One set of No. 40 stood the test, but No. 29 failed. No. 18 was not repeated.

† R 20/4, rotten at this date in 1939; M denotes missing, viz. rotted away.

‡ Stood over 200 lb. on spring balance. Figure given is for weights applied slowly.

§ Gave 155 and 285, latter omitted from average.

‖ The mixture was used in error for the concentrate used in Table I.

the full run from 1938. Nets, twines and ropes all confirm the results obtained by Fillon (1925) with cotton nets and show that a previous cutch treatment is an improvement before the copper soaps or coal distillates are applied. It is advantageous to use tar warm, especially with ropes, as better penetration is secured. There is a great benefit from using copper soaps with the latter as against using the distillates alone or after cutch. To fix the cutch by Olie's method before applying the copper soap mixture seems an advantage with naphthenate, though not with oleate. The best preserved trawl twine and yacht manila had a final strength 44–48 % of the initial and received oleate, resinate or naphthenate mixtures after cutch; but the oleate was good for the twines especially as the penetration is poor with thick ropes.

We were unable to test breaking loads beyond 300 lb., but it is remarkable that every copper soap treatment gave an average value exceeding this limit on the 700 lb. six-thread Gourock manila rope, and only two samples in set A broke below 300 lb. The untreated rope was at this stage valueless, having only 3 % of its initial value as against over 43 %.

These results confirm and extend the earlier tests (Atkins & Purser, 1936) with 2 in. ropes of hemp, manila, sisal and coir, which showed that the preservatives are about equally good on the various fibres. The present tests were accordingly done on manila only.

Taken in conjunction with the previous results, one may conclude that while for ropes of small diameter resinate, oleate and naphthenate copper soaps are all good, yet for thick ropes the more costly naphthenate has the advantage of better penetration.

It is often said that a combination of wet and dry conditions is more destructive to nets and ropes than continual wetness. Table I shows the results of immersion of ropes for a year, and subsequent storage dry. Tables II and III give results where the specimens were out of water every tide, the total immersion being for 14 months. The two sets are not strictly comparable, but the treatments common to both had the final percentage strengths indicated, respectively, oleate in tar, 20 and 38 %; Lever's mixed copper soap, 31 and 30 %; Cuprinol, 44 and 33 %; Cuprinol, British Columbia grade, 35 and 29 %. The results are therefore inconclusive, at least for preserved materials, for which the leaching out of the preservative may offset the advantage, if any, of remaining wet. With trawl twines the evidence was also indecisive and even contradictory. It would be necessary to take a large number of samples for each treatment and to pay special attention to the humidity of the air, viz. the moisture in the fibres, in order to decide this point. Since in practice treatments have to be carried out when required no such differences were taken into account.

The treatments of the nets, which perished during wet storage late in 1938, were repeated early in 1939 and the samples were immersed as before from March 6 to September 11, 27 weeks. Those which survived were then laid out on a gravel path for 14 weeks. The treatments which remained sound are shown in Tables II and III. In addition certain new treatments stood the test as follows:

No. 54, cutch treatment, then in bichromate at 40° C. for 1 min. with sulphur dioxide bubbling through.

No. 71, green Cuprinol (copper naphthenate solid 12% Cu) in tar, 1 lb. to 1 gal., also 74, as 71 after cutch and 75, after Olie's treatment.

No. 78, copper naphthenate (Brent) with 8% Cu in 95% alcohol, one set good, but (No. 79) both good after cutch.

No. 82. As 78, 1 lb. to 1 gal. tar, after cutch or (83) Olie's treatment.

Nos. 84–87, cutch treatments, including Olie's, followed by dilutions of concentrated Cuprinol with petrol, all failed; this solvent is clearly unsuitable. Similar treatments without cutch also failed.

A series of exposures was also begun to test the suggestion kindly sent by Mr J. S. Tait of Vancouver, B.C., that the addition of boiled linseed oil improved the value of Cuprinol. The samples were immersed on June 29 and had 10½ weeks in the basin before removal, on account of war, on September 11. Those which survived then had 14 weeks on the gravel path. The untreated control was rotten after the water test. After the air exposure the following were in poor condition: No. 89, linseed oil; No. 98, green Cuprinol; No. 99, Cuprinol, British Columbia grade; No. 92, Cuprinol B.C. with 10% linseed oil; No. 94, Cuprinol with 10% linseed oil; No. 95, ditto with 20% linseed oil. No. 96, Olie's treatment, followed by linseed oil, gave one good and one poor net.

The following remained sound: No. 90, cutch, then linseed oil, and No. 91, as 90 with a further boiling in cutch; No. 93, 20% linseed oil in Cuprinol, B.C. grade; No. 97, copper oleate in tar 1 lb. to 1 gal.

SUMMARY

Manila ropes and trawl twines, and cotton nets, have been immersed in clean sea water for periods up to 14 months, some with alternate wetting and drying, some wet all the time, but in both cases accompanied by storage in air after immersion. All the untreated specimens perished, but the 700 lb. manila ropes retained at least 40% of the original tensile strength in every case in which a copper soap had been included in the treatment. A previous treatment with cutch is a considerable advantage as shown by the 500 lb. manila ropes and trawl twines, though cutch alone is worthless. Coal tar distillates after cutch are, however, more useful than when used alone, and their mixture with copper soaps gives excellent results. Cutch fixed with bichromate gives tolerably good preservation on nets up to 6 months, but is a poor preservative for trawl twines and ropes which are expected to have a long life. The choice of the copper soap used depends upon the material to be preserved. Naphthenate is the most soluble and penetrates thick ropes excellently; on fine nets it leaches out easily. Oleate is cheaper, but its penetration is less; it does very well with tar on twines and nets. Resinate is the least soluble,

but is useful with creosote oil or tar. On account of their greasy nature naphthenate and oleate act as lubricants in ropes.

Immersion tests on other preservatives, kindly suggested by Dr S. G. Barker, are to be begun.

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A NOTE ON THE DETERMINATION OF VERY SMALL QUANTITIES OF HYDROXYLAMINE BY BLOM'S METHOD

By H. Barnes, B.Sc., B.A.

In a study on the possible occurrence of hydroxylamine as an intermediate compound in the oxidation of ammonium salts to nitrites in sea water (see Cooper, 1937), the method of Blom was tried in order to estimate the hydroxylamine. The method consists in the oxidation of the hydroxylamine by a solution of iodine in acetic acid, removal of the excess of iodine with *N*/10 thiosulphate, and estimation of the nitrite so produced by the Griess method; the method has been used by several workers for the estimation of small amounts of hydroxylamine in biological material.

The method had not been used for such small amounts of hydroxylamine as would be expected in sea water, and it was therefore tested at high dilutions. It was found that nitrite itself, however, could not be recovered after treatment with the above reagents, and it was noticed that in many cases a precipitate (probably sulphur) was produced, and a distinct smell of hydrogen sulphide was often apparent. It was thought probable therefore that the loss was due to the interaction of the nitrite or the red dye with the thiosulphate used for removing the excess of iodine. This view was confirmed by noting the removal of the red colour, the appearance of a precipitate, and the smell of hydrogen sulphide when a solution of thiosulphate was added to a solution of the dye.

Experiments were therefore set up to determine the amount of thiosulphate that could be present in excess without interfering with the estimation. Varying amounts of thiosulphate were added to a known concentration of nitrite (30 mg. per $m.^3$), and the colour produced on adding the Griess reagents was then compared with that obtained without the addition of thiosulphate. The solutions were placed in 100 ml. cylinders and matched in the usual manner. The results are shown in Table I.

It is clear from the Table that when the concentration of the thiosulphate is greater than about 1 mg. per litre the nitrite estimation is inaccurate, and that when the concentration is about 6 mg. per litre only 50 % of the nitrite is found. Taking this latter figure for illustration, although it represents a very considerable excess when compared with nitrite present, it is very small in terms of *N*/10 thiosulphate used in the removal of iodine in the normal process. According to these results, if in estimating the strength of solution of hydroxylamine, equivalent to a nitrite production of 30 mg. per $m.^3$, 3/500 of a ml. of thiosulphate more than that required to remove the excess iodine were added, only half the nitrite actually produced would be found. It is clearly very difficult to add the thiosulphate within these limits.

Table I

Conc. of thiosulphate (mg. per litre)	Reading of control	Conc. of thiosulphate (mg. per litre)	Reading of control
0·025	100	1·5	82
0·05	100	2·0	71
0·15	100	3·0	66
0·2	100	4·0	62
0·25	100	5·0	56
0·3	100	6·0	53
0·35	100	7·0	50
0·4	95	8·0	51
0·45	99	9·0	42
0·5	100	11·0	41
0·75	100	14·0	32
1·00	100		

Conc. of nitrite 30 mg. per m.^{3}

I should like to thank Dr Cooper for suggesting this work and for his continued interest during its progress.

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A NOTE ON THE CHANGES IN WATER CONTENT OF THE LOBSTER (*HOMARUS VULGARIS* M.-EDW.) DURING MOULT

By A. G. Lowndes and N. K. Panikkar

From the Plymouth Laboratory

One of the lobsters kept in the tanks of the Plymouth Laboratory moulted on the night of 20 August 1940. This opportunity was taken to examine the changes in water content and osmotic pressure of the freshly moulted as compared with unmoulted lobsters. Though only one recently moulted individual has been examined, the results seem to be worthy of note since so far as we are aware no data are available on the subject in regard to *Homarus*. The biology of moulting among lobsters has received the attention of many investigators (vide Herrick (1895) and Drach (1939) for summary and literature).

The water content was determined by distilling the fresh lobster together with a known weight of sea water under xylol. This method is a modification of that of Dean and Stark which one of us (A. G. L.) has tried on a number of animals and found to give accurate results. The density was estimated by a modification of a method previously described (Lowndes, 1938). Osmotic pressure of blood and the external medium were measured by Baldes's (1934) modification of the Hill thermoelectric technique, as employed by one of us (N. K. P.) in the study of prawns and other crustaceans. The water content of the moulted skin was calculated from its dry weight taken after dehydrating in a hot air oven for 48 hr. at 105° C. Osmotic pressure was measured 17 ± 6 hr. after moult; density and water content after 34 ± 6 hr. The temperature of sea water at which the density was estimated was 17° C. Both lobsters were 4–5 years old as judged by Elmhirst's (1930) growth curve. The results obtained are given in Table I.

The higher values for density and sinking factor are just what one would expect from the highly calcified exoskeleton in the lobster of late intermoult phase. *Homarus* is a stenohaline invertebrate which is isotonic with its surroundings and it is interesting to note that more or less the same degree of osmotic equilibrium with the surroundings is observed in less than one day after moult. Hence, if there is any rapid rise in osmotic pressure prior to moult, as has been observed in some Crustacea, this anisotonicity disappears soon after moulting. The values of water content indicate that a lobster of fresh weight 100 g. loses about 28 g. when its skin is shed, and that it absorbs about 47 g. of water within 34 ± 6 hr. after moulting. Thus an increase in weight of 19 % is observed in so short a period. Elmhirst (1930) has shown that this increase in weight during the post-moult phase may be as high as 38 %, but it may be noted that the post-moult phase during which it takes place lasts from 15 to 30 days.

TABLE I

	Density g. per ml.	Sinking factor*	Osmotic pressure of blood % NaCl	Osmotic pressure of medium % NaCl
Soft lobster, ♀	1·077	1050	3·410	3·338
Hard lobster, ♀ (late intermoult)	1·158	1128	3·403	3·346

Lobster	Volume ml.	Gross weight g.	Wt. of water g.	% water	% water including moulted skin
Soft: ♀ after moult	110·02	118·47	97·43	82·24	77·30
Moulted skin alone	—	45·14	29·04	64·33	
Hard: ♀ late intermoult	136·13	157·61	105·16	66·82	66·68

$$* \text{ Sinking factor} = \frac{\text{Density of organism}}{\text{Density of medium}} \times 10^3.$$

It may be seen from these figures that:
The amount of water in a lobster of dry weight 33·32 g. after moult

$$= \frac{33{\cdot}32 \times 77{\cdot}3}{22{\cdot}7} = 113{\cdot}5 \text{ g.}$$

Therefore the amount of water absorbed = 113·5 − 66·68 = 46·82 g.

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THE OCCURRENCE AND BREEDING OF *SAGITTA ELEGANS* VERRILL AND *SAGITTA SETOSA* J. MÜLLER IN PARTS OF THE IRISH SEA

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(Text-figs. 1–2)

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INTRODUCTION

Investigations in recent years have recognized the fact that certain species of *Sagitta* are associated with particular bodies of water, and that when these species are swept by currents into foreign water masses they exist there for limited periods of time. The relatively large size of *Sagitta* makes them convenient to handle and increases their value as representatives of their respective localities.

There has been no previous prolonged examination of the *Sagitta* in Liverpool Bay with respect to the present classification of the species. Russell (1935, p. 323) has recorded *S. setosa* J. Müller and *S. elegans* Verrill from the north of Ireland and from Port Erin. The present investigation has demonstrated that the *Sagitta* present in Liverpool Bay are *S. elegans* and *S. setosa*. The latter species had not been recorded before from this region. During the period of my study it was the dominant form in these coastal waters.

The work of Russell (1932*a*, *b*, 1933*a*, *b*, 1935), Meek (1928), Wimpenny (1936), and others, on the life history and distribution of the species of *Sagitta*, has placed emphasis on the importance of data from other areas. As a result of their work and of the discovery of *S. setosa* in Liverpool Bay, the problem on which this paper is based is the distribution and relative abundance of *S. setosa* and *S. elegans* in parts of the Irish Sea, and the number and periodicity of their reproductive cycles.

I am indebted to Prof. J. H. Orton for general supervision of my work and for helpful advice. Mr F. S. Russell verified the maturity and classification of a number of specimens sent him. Dr Jenkins, chairman of the Lancashire and Western Sea Fisheries Committee, kindly enabled me to collect from the fishery cutters in Liverpool Bay. Mr Nicholson, operator of the cutter at Liverpool, assisted by making many tow-nettings for me. Dr R. J. Daniel, Director of Port Erin Laboratory, and members of the staff at that station, made it possible for me to study plankton samples from Port Erin. Finally, I wish to thank the various other persons who have aided me in the collection and preparation of this material.

COLLECTING STATIONS AND APPARATUS

Collections were obtained once a week from each of three localities, namely: Pier Head, Liverpool Docks, Liverpool; the outer Mersey Channels; and Port Erin harbour, Isle of Man.

The Pier Head station at the mouth of the Mersey River was selected because it was easily reached, would furnish a plankton sample from estuarine conditions and *Sagitta* were known to be available from there. Two nets were used: a ½ m. stramin net sunk with a weight to an approximate depth of 6 m.; and a fine silk net (104 meshes per inch) ½ m. in diameter suspended just beneath the surface. Both nets were allowed to remain in an ebb tide for ½ hr. The tide at this point flowed at the rate of 1 knot.

The outer Mersey Channels include an area which extends 14 miles into the Bay from the mouth of the river. The samples were usually taken 7 or 8 miles from the mouth of the Mersey in the Queen's and Crosby Channels. Two nets were used: a ½ m. stramin net towed at a depth of 6–8 m. (occasionally in shallower water it was towed just 3 or 4 m. deep); and a ½ m. silk net (64 meshes per inch) towed just below the surface. The period of towing was ½ hr. at the rate of approximately 1 knot.

In both the above stations the depth of the water and the diurnal vertical migration of the *Sagitta* were the chief factors considered in selecting the depth at which the stramin nets were towed. The vertical movement of *Sagitta* has been recorded by several investigators (Michael, 1911; Bigelow, 1924; Russell, 1935). Several tests were made to determine the extent of this movement in the comparatively shallow waters of the outer Mersey Channels. The surface towings made during these trials contained relatively few *Sagitta*. Below a few metres it was difficult to determine at what depth the animals were most numerous. I believe this rather scattered distribution was due to the continual mixing of the water layers by the strong tides in these channels. The water was always heavily silted and of low transparency. I have found on several occasions at the Pier Head, where the water was even more muddy and turbulent than farther out in the Bay, that I have captured almost equal numbers in both nets during one haul.

The third regular station from which weekly collections were obtained was the harbour at Port Erin, Isle of Man. Here two silk nets, 36 cm. in diameter with 64 and 125 meshes per inch respectively, were towed across the harbour behind a rowing boat. These towings were taken just below the surface for a period of 20 min.

STAGES OF MATURITY

Sagitta is hemaphroditic with ovaries in the posterior portion of the body cavity and testes extended in two narrow bands, one on either side of the tail cavity. The testes mature first, filling the tail cavity with clusters of sperm cells in various degrees of development. The early indications of maturity in the ovary are an increase in size accompanied by an apparent increase in the number of eggs. In the final stage the eggs increase rapidly in size, a few at a time becoming distinctly larger than the remainder in the ovary.

Several investigators have recognized stages in the development of the germ cells which may be determined visually in the whole animal. Kramp (1917, p. 37) and Wimpenny (1936, p. 17) have each defined four main stages in the development of the gonads. These stages, though differing in some details, are very similar. Russell (1932*a*, p. 134) has simplified Kramp's divisions, describing three stages of maturity in place of four. Russell's descriptions have appeared to me to be the more suitable and I have classified my specimens according to the stages recognized by him.

These stages, designated as I, II and III, represent progressive sexual development from the young immature individual, through an intermediate form, to the adult and fully mature animal. Quoting from Russell (1932*a*, p. 134): "Stage I included all the youngest *Sagitta* in which not a single sperm mother cell was visible lying loose in the tail cavity. Stage II ranged between those individuals with the first appearing spermatocytes and those in which the tail segment was packed with spermatocytes and spermatozoa, but in which the ovaries, while appearing evident, showed little sign of swelling eggs. Stage III contained those individuals in which the ovaries were fully ripe or ripening" (e.g. contained one or more eggs very much enlarged).

TREATMENT OF MATERIAL

Sampling. The *Sagitta* were placed in 10% formalin shortly after capture. Later the total number was counted, the stage of maturity determined and each specimen measured. If the weekly collection exceeded fifty specimens a representative fraction containing about forty individuals was separated for maturity determinations and measuring. The measurement of the body length did not include the caudal fin which is delicate and easily damaged.

Staining. In order to ascertain accurately the maturity of the sperm and ovary it was necessary to stain the animal. A successful method for staining large numbers of *Sagitta* was as follows: groups of specimens (20–30) were transferred gradually from the 10% formalin to 75% alcohol and placed in small glass tubes ($\frac{1}{2} \times 2$ in.). Three or four drops of a concentrated solution of borax carmine were added to the tubes and gently mixed. A day later the borax carmine solution was poured off and replaced by 75 % alcohol and one drop of concentrated hydrochloric acid. The *Sagitta*, now a light red, were kept in the acid alcohol until by inspection the body wall appeared translucent (20–30 min.); the ovary and testes were still red. At this point the acid alcohol was replaced with 75 % alcohol. A second change was sometimes given if continued fading was noticed. The advantages of this method are that large numbers of specimens can be handled with a minimum of effort, and little or no shrinkage or distortion of the *Sagitta* will take place.

PORT ERIN *SAGITTA*

Port Erin material, 1928–34. The data for *S. elegans* at Port Erin for 1928–34 inclusive, are shown in Table I and Fig. 1. The weekly catches during these years had been combined in fifty-two grouped samples. Each sample contained the plankton collected in that week for all of the years under consideration. These weekly catches have, for brevity and clarity, been graphed in monthly units.

TABLE I. PERCENTAGES OF *S. ELEGANS* GROUPED IN STAGES I, II AND III

Taken from Port Erin 1928–34

Month	Stage I %	Stage II %	Stage III %	No. counted from sample
Jan.	4	69	27	89
Feb.	5	49	46	120
Mar.	14	41	45	46
Apr.	27	50	23	105
May	52	47	1	84
June	60	40	0	122
July	82	16·5	1·5	209
Aug.	91	9	0	192
Sept.	94	3	3	135
Oct.	98	2	0	120
Nov.	78	22	0	108
Dec.	37	63	0	128

Fig. 1 presents the relative occurrence of the three maturity stages and their relative percentage frequency. Length (mm.) is plotted on the ordinate of the graph and percentage frequency on the abscissa. Stages I, II and III have been treated separately in each month. The absence of a particular stage in the graph for any month is evidence that such a stage is scarce or wanting in the plankton for that period. A similar graph (Fig. 2) has been drawn of the weekly plankton catches of *S. setosa* taken in the outer Mersey Channels during 1937. The appearance of *S. setosa* at Port Erin and *S. elegans* in the outer Mersey Channels was too sporadic to graph in this manner; however, for a short period in the year they became relatively abundant (Pierce & Orton, 1939).

The graph of *S. elegans* from Port Erin (Fig. 1) shows that the main spawning period is during the months of January, February, March and

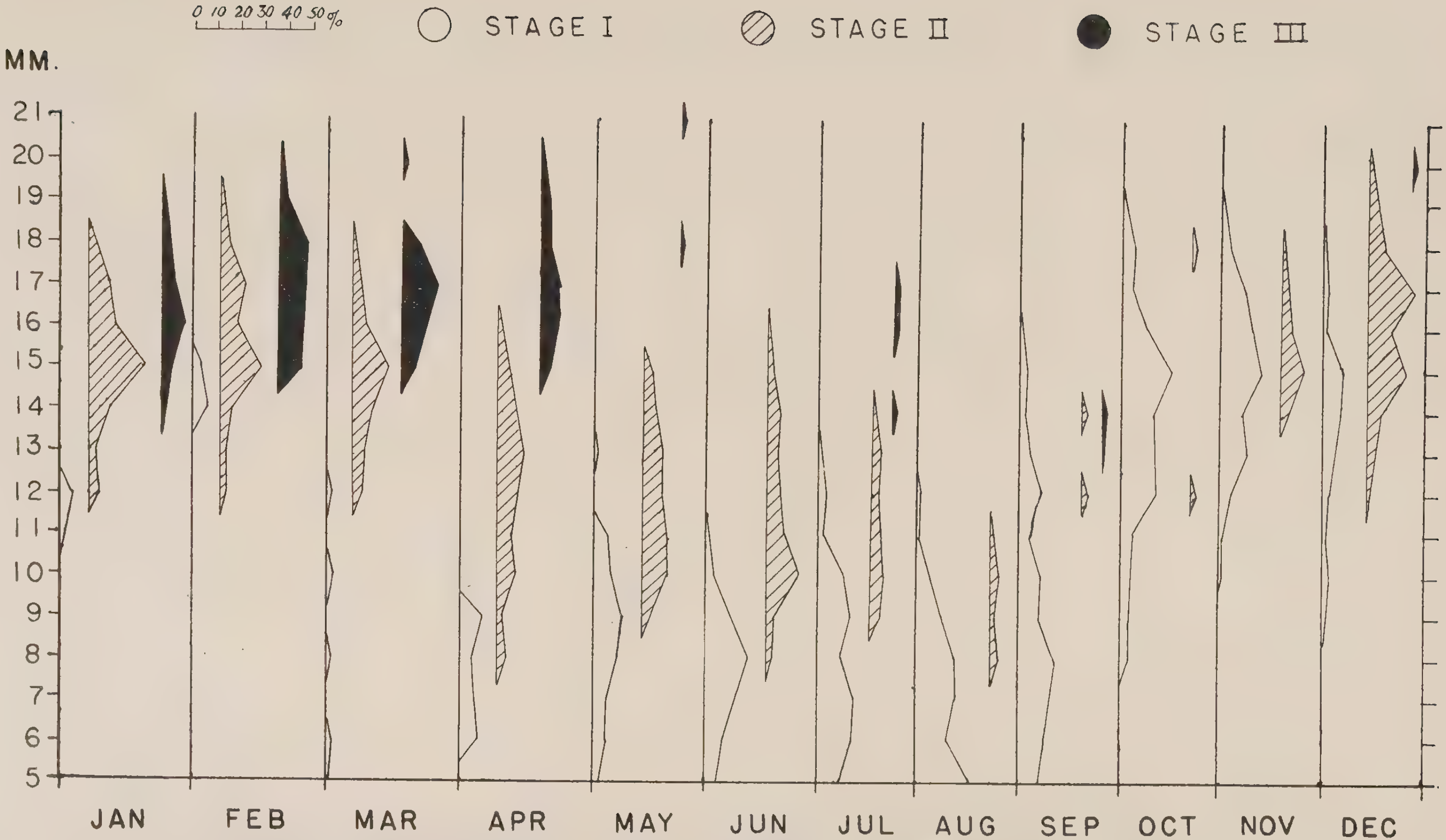

Fig. 1. *S. elegans* caught at Port Erin during 1928–34. Length (mm.) is plotted on the ordinate of the graph and percentage frequency on the abscissa. Stages I, II and III have been treated separately for each month.

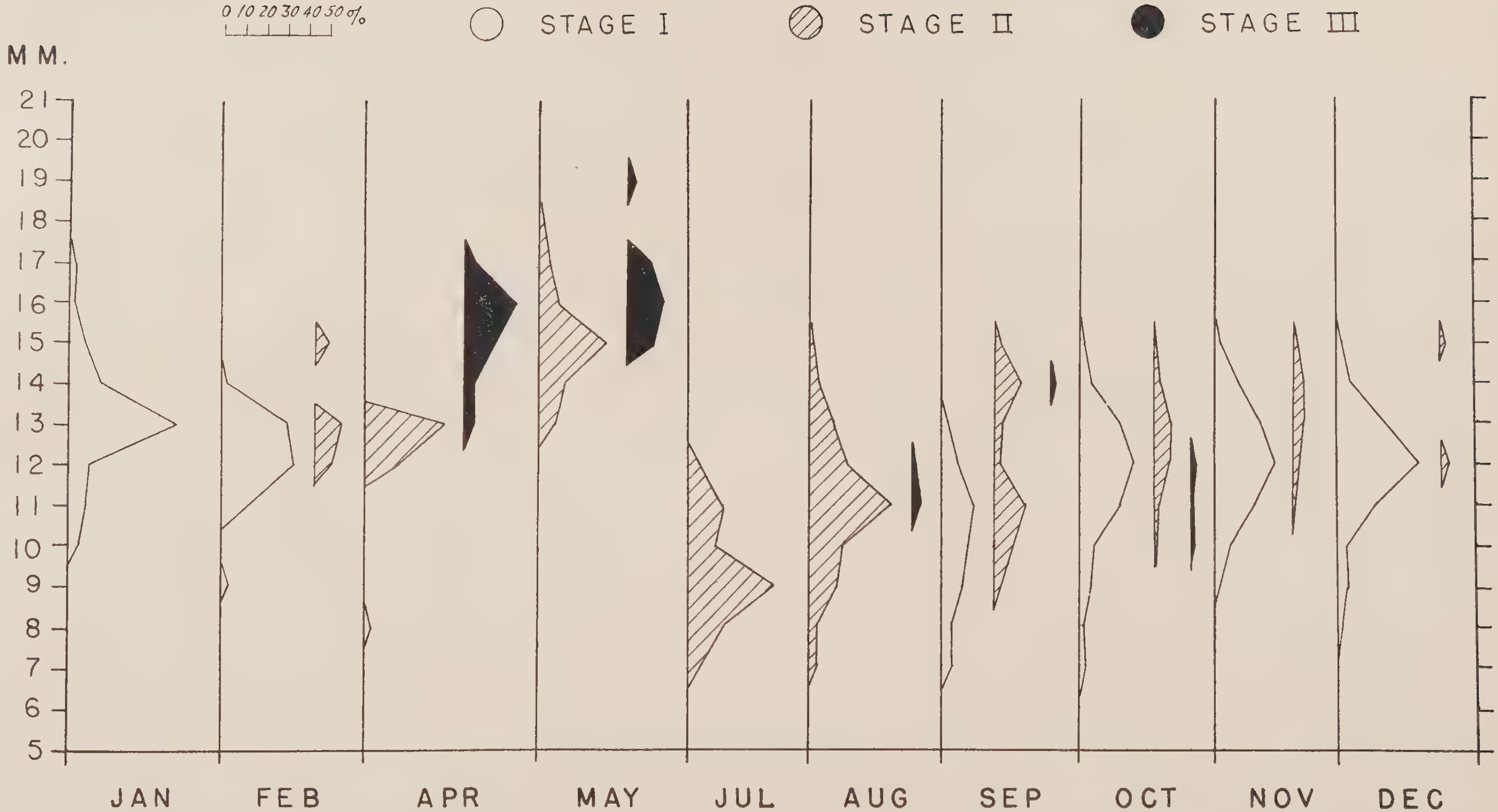

Fig. 2. *S. setosa* caught in the outer Mersey Channels in 1937. Length (mm.) is plotted on the ordinate of the graph and percentage frequency on the abscissa. Stages I, II and III have been treated separately for each month.

April. The stage III *Sagitta* increase from less than 1 % in December to 27 % in January, 46 % in February, and 45 % in March, and decrease to 23 % in April and 2 % in May. Table I contains the complete annual percentages for all three stages. A few mature individuals, as indicated by the small black areas on the graph, were found at scattered intervals throughout the grouped years. Their numbers in these instances were too small to permit recognition of a definite spawning season.

The first young appeared in large numbers in April and from then onward they increased, outnumbering the individuals in the other two stages through November. In November, stage II became noticeable (22 %) and increased to 63 and 69 % in December and January respectively. As indicated above many of the *Sagitta* have become mature by January which thus completes the breeding cycle for the year.

Port Erin material, 1937. Table II of the *S. elegans* collected at Port Erin in 1937 agrees closely with the grouped data for the years 1928–34. A scarcity of specimens in March and October gave few records for those months; however, this does not seriously impair the sequence of the data in the table.

In this year the spawning season began a month later (February) when 18 % of the specimens were mature, and extended through May. The stage II individuals attained their maxima of 73 % in January and 80 % in February, as in 1928–34. The October data are scarce, but the abundance of stage II in November (68 %) indicates that the *Sagitta* began maturing in October, developing into mature individuals in numbers in February and the succeeding months. This completes their annual breeding cycle.

TABLE II. PERCENTAGES OF *S. ELEGANS* GROUPED IN STAGES I, II AND III

Taken from Port Erin in 1937

Month	Stage I %	Stage II %	Stage III %	No. counted from sample
Jan.	23	73	4	66
Feb.	2	80	18	90
Mar.	—	—	—	—
Apr.	4	35	61	138
May	33	33	34	126
June	57	36	7	275
July	94	4	2	87
Aug.	85	12	3	36
Sept.	94	3	3	29
Oct.	89	11	0	9
Nov.	32	68	0	49
Dec.	7	93	0	57

Discussion. In view of the possibility of discrepancies in sampling such a shifting population the close agreement of the data from the grouped samples from 1928–34 and for the single year 1937 is noteworthy. The maxima and minima for stages I, II and III fall in every case within a month of each other. A comparison of Tables I and II reveals a gradual increase and diminution of the individuals in each group. There are no exceptionally large populations in any month which are not in agreement with the appearances of the *Sagitta* in both the preceding and following months. These are important features, because they substantiate the validity of the sampling methods and support the conclusion that there is but one main season of reproduction for *S. elegans* in the Port Erin region.

Additional collections of *Sagitta* were examined from Port Erin for the years 1935 and 1936. The data from these samples are in essential agreement with the material already discussed and permit of the same conclusions. This information appears useful chiefly as an additional check on the 1928–34 and 1937 breeding cycles and will not be discussed further here.

Russell's data (1932*a*) have indicated four main breeding periods a year for this species in the Plymouth area. These occur in February, May, June-July and September. A comparison of his results with mine reveals that a spawning period in February coincides. After that date and through September no additional breeding periods were identified, as recorded by Russell. In October, November, December, January and February the stages in development appear very similar.

Wimpenny (1936) collected *Sagitta* from the south-west portion of the North Sea. He places one of the breeding periods of *S. elegans* between January and February, which agrees with Russell's and my results; also his data indicate a spawning period between April and August and another tentative breeding in October, which in general is similar to the reproductive periods at Plymouth. In contrast the one long spawning period from January-February to April-May evidenced by my results from Port Erin does not correspond completely with the observations of either Wimpenny or Russell. The great discrepancies appear to lie in the long breeding period from winter to spring of the Port Erin species and the lack of another in the succeeding summer.

Liverpool Bay *SAGITTA*

Outer Mersey Channels. The *Sagitta* collected in the outer Mersey Channels in 1937 were with few exceptions *S. setosa*, and the graph (Fig. 2) includes only that species. During January and February this species comprised 95 and 58% respectively of the total number of the two species. Owing to a mishap to the fishery cutter the March data are missing. For the remainder of the year the entire catch consisted of *S. setosa* (Pierce & Orton, 1939).

The first spawning of the year, as indicated in the graph (Fig. 2) occurred in April, May and probably June. During April stage III amounted to 48% of the catch and in May 40% (Table III). In June the presence of the alga *Phaeocystis* in great quantities plus large numbers of the ctenophore *Pleurobrachia pileus* rendered the plankton nets almost useless; moreover, the *Sagitta* themselves appeared very scarce.

Following the April-May spawning the young produced in June must have started maturing rapidly, because all individuals collected were in stage II in the latter part of July when the first *Sagitta* for that month were obtained in the outer Mersey Channels. By August the first mature individuals from the April-May spawning appeared. The occurrence of these stage III forms, followed by the appearance of numerous stage I specimens, is evidence of a second breeding period despite the scarcity of mature *S. setosa*. During October, November and December stage I continued to increase in relative frequency and by January comprised 100% of the catch. The testes started maturing during February and large individuals ready for spawning appeared in April and May, completing the annual cycle.

It is to be noted that the stage II individuals from February to May (first brood) are larger than the stage II specimens taken between July and November (second brood). The former attained an average length of 13·4 mm. in February, 13·2 mm. in April and 16·0 mm. in May. In the second generation the male stage was first noted in July, when an average length of 9·4 mm. was recorded. By August this had increased to 11·1 mm., in September to 12·0 mm., to 12·6 mm. in October and 13·1 mm. in November. The stage II specimens had almost vanished from this locality by December. The increase in body length found during the colder months (i.e. February, April and May) agrees with the records of Russell (1932*a*) and Bigelow (1924), who have noted a relation between length and temperature; the greater length being found during the colder months. Similar conclusions hold for the stage III *S. setosa* taken; however, in view of the small numbers collected in the summer, details of average length have been omitted.

Pier Head samples. Throughout the year 1937 weekly plankton samples were collected from a pier at the mouth of the Mersey River. Usually the numbers of *Sagitta* caught here were small (Table III). These samples are valuable largely because they confirm the results of the collections taken farther out in the Bay (Fig. 2).

It is of interest to note that during February when mixed catches of *S. setosa* and *S. elegans* were caught in the outer Mersey Channels a similar mixed catch was obtained from the Pier. In March, when the fishery cutter was disabled, towings from the Pier were obtained. The first on March 18 contained a single *S. elegans*. The second sample on March 30 contained nine *S. elegans*; no *S. setosa* were caught during that month. This record agrees with the record of *S. elegans* obtained in Morecambe Bay in March. The remainder of the year produced only *S. setosa*. Collecting at Pier Head was discontinued after the first week in December 1937.

Comparison with previous data. Russell (1932*b*) at Plymouth finds at least six generations of *S. setosa* per year. There is a greater similarity between his results and mine than may at first appear. He records a generation in April and May, and another in June. I believe, in the Liverpool region, that *S. setosa* spawn continuously for these three months, thus combining two of his breeding seasons in one. There was a dearth of mature individuals in July, but there is evidence for a breeding season in August extending into September. This is also similar to the July-August and September spawnings indicated by Russell. Little evidence was found for a breeding season in October and February, such as he records.

Wimpenny (1936) finds two spawning seasons for *S. setosa* in the south-western portion of the North Sea. The first was in April and the second in July. The earlier season corresponds to that indicated by Russell's data and my own. The July spawning agrees with Russell's records for a brood in July-August and appears to correspond with my evidence for a spawning in August.

Additional Samples

Morecambe Bay. A number of plankton samples were obtained from Morecambe Bay during March and April 1937. The number of *S. setosa* taken is listed briefly as follows: March, stage I, 1 specimen; stage II, 86 specimens; April, stage II, 6 specimens; stage III, 5 specimens.

A graph of the March 1937 collection of *S. setosa* would readily fit between the February and April results from the outer Mersey Channels (Fig. 2), as is evident from the above data. The small April collections agree with those taken in the outer Mersey Channels in the same month.

The number of *S. elegans* found was: March, stage II, 34 specimens; stage III, 105 specimens. None were taken in April. These data agree very well with the 1928–34 and 1937 records from Port Erin.

Anglesey. During the first week in May a trip was made around Anglesey on Board the Lancashire and Western Sea Fishery steamer *Charles MacIver*. Although plankton towings were made completely around the island and in the Menai Strait, *Sagitta* were obtained only from the northern coast in the vicinity of Point Lynas and Moelfre. Relatively few *Sagitta* were taken even here. The total number caught was fifty-eight; of these thirty-eight were *S. elegans* and twenty were *S. setosa*. The large majority of the thirty-eight *S. elegans* were stage III individuals; the remainder were stage II. These specimens are similar both in length and development to the *S. elegans* taken in April and May 1937 from Port Erin (see also Fig. 1). In both species there is a preponderance of stage III forms, indicating a spawning.

There were thirteen stage III specimens as compared to the seven stage II forms among the twenty *S. setosa* caught. These data, though scanty, agree with the May records of the species taken from the outer Mersey Channels some 50 miles east.

Range of *S. setosa*

S. setosa and *S. elegans* are evidently distributed along the west coast of England and Wales from Morecambe Bay to Anglesey, for at least portions of the year. In view of Russell's records (1936, Fig. 6) of *S. setosa* and *S. elegans* from the vicinity of Land's End, and additional records of these two species from the north coast of Ireland, it appears probable that they extend along the entire coast of England and Wales bordering the Irish Sea.

Formerly, *S. setosa* was believed to be indigenous only in the English Channel, North Sea and neighbouring waters. Also it appears that the *Sagitta* at Port Erin, Morecambe Bay, outer Mersey Channels and Anglesey are members of a more or less related population whose breeding periods for each species are approximately the same.

NUMBERS OF *SAGITTA* COLLECTED PER MONTH

Table III records the number of *Sagitta* taken per month for the year 1937 at Port Erin, Pier Head and the outer Mersey Channels. Plankton catches from Port Erin taken with a surface tow-net as a rule produced the greatest number of specimens during the summer. This was the period when the young forms were comparatively numerous; later, as winter approached, the catches decreased somewhat, reaching a minimum in January and February. These results are merely indications of the actual quantity of *Sagitta* present during this time. Larger collections taken with nets at greater depths will be required before even general quantitative conclusions may be drawn.

TABLE III. TOTAL NUMBER OF *SAGITTA* CAUGHT PER MONTH IN 1937

Month	Port Erin	Pier Head	Mersey Channels
Jan.	66	2	69
Feb.	205	19	72
Mar.	—	10	—
Apr.	138	9	75
May	126	3	90
June	590	2	7
July	87	9	57
Aug.	36	4	90
Sept.	34	11	140
Oct.	—	60	1399
Nov.	269	126	377
Dec.	57	16	70

Certain general trends in the increase and decrease of the *Sagitta* in the outer Mersey Channels were more discernible. In June 1937 *S. setosa* had practically vanished from this region. A marked scarcity continued until the latter portion of July when fifty-seven specimens were secured. The noticeable increase in September was due to the presence of the young *Sagitta*. This increase reached a peak in October. After October, although *S. setosa* was present in numbers, no very rich hauls were obtained. The trend from then onward appeared to be one of decrease which would result in a similar scarcity the following summer.

SUMMARY

An investigation has been made of the species of *Sagitta* present in parts of the Irish Sea, their relative occurrence, and the annual number of reproductive cycles of each species. Three stages of maturity as described by Russell (1932*a*) have been used. To determine the maturity the *Sagitta* were transferred to a solution of 75 % alcohol and stained with borax carmine.

S. elegans Verrill and *S. setosa* J. Müller were collected in Liverpool Bay and in Port Erin harbour, Isle of Man. The former species was predominant at Port Erin. The coasts of Liverpool Bay have been added to the permanent range of *S. setosa*.

At Port Erin there appears to be but one chief spawning period for *S. elegans* which extends generally from January through May. Following May the young forms appear in numbers and gradually mature through December to repeat the annual cycle.

There appear to be two main breeding seasons for *S. setosa* in the outer Mersey Channels. The first and most noticeable begins in April and extends into June. There is evidence for a second breeding period in August.

Both species were found in the samples taken from Morecambe Bay and Anglesey. The stage of maturity of these agreed with the *Sagitta* caught at the same time at Port Erin and Liverpool.

The total monthly catches of *Sagitta* from Port Erin and the outer Mersey Channels have been recorded.

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OYSTER REARING ON THE RIVER YEALM

By Douglas P. Wilson, M.Sc., F.R.P.S.

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In a recent issue of this *Journal* Dr Hughes (1940) published records of a successful attempt to rear oysters in tanks on the River Yealm during the summer of 1939. In this paper he referred to plans for the following year, and it therefore seems desirable to record a few notes on the 1940 season.

Since Dr Hughes left Plymouth I have kept in touch with Mr J. Kingcome, the owner of the fishery, and from time to time have visited his tanks and proffered technical advice. In this I have had the willing co-operation of Mr H. A. Cole of Conway whenever his opinion has been sought. Mr Kingcome has most readily adopted suggestions we have made, and it is to his careful attention and unremitting work that the successful results here recorded are due.

FURTHER HISTORY OF THE 1939 SPAT

Dr Hughes mentioned that in August 1939 some of the larger spat were detached from the tiles and placed in cages in the estuary of the river. At the time of writing (mid-October 1940) these range from 4·5 to 8 cm. in diameter. The remainder of the spat spent the winter on the tiles in tank C (Hughes, 1940, Fig. 1), being given almost daily changes of water from the estuary. They were detached from April to July 1940 and placed like the others in wire-meshed cages just above low-water mark spring tides. These oysters have by no means reached the size of those which have been out since August 1939, the size range being 2–4 cm. in diameter.

It is difficult to make a reliable estimate of the number of young oysters now in the cages, all of them from the 1939 spatfall. Mr Kingcome believes there are 20,000–30,000; this figure seems to me to be reasonable. They are in splendid condition and there is every prospect of eventually bringing them to marketable size.

THE 1940 SPATFALL

Rearing has again been successful under quite different weather conditions. In 1939 there was much rain and heavy cloud, but this year has been outstanding for an almost unbroken period of fine weather from May to September, although heavy rain occurred once or twice during the breeding period. At Conway spat settlement has occurred over a wide range of meteorological conditions (Cole, 1936). As before, tank A was used for breeding and the water in it enriched by Cole's method (1938). The bottom arrangements were similar to those of last year, the stock oysters lying on slatted trays covered with large flat tiles. Smaller flat tiles were leant up against the sides of the tank at the bottom, while others were propped against one another in rows of inverted V's. In addition some broken curved tiles were piled on the bottom. The tank was flooded from the estuary on June 12 and enrichment with crab meat began a week later. Larvae were first noticed on June 23 (the same date as last year) but may have been present earlier. About this time several bundles of curved tiles were suspended a short distance below the surface. It had been the intention to hang in this way the majority of the limed tiles used for spat collection but unfortunately there was at the time a shortage of curved ones, and for this reason many flat tiles, which are not easy to suspend, were placed on the bottom. Mr Kingcome also scattered a large number of oyster shells after the tank had been filled: most of them reached the bottom white sides uppermost. Some bundles of shells were hung in net bags at the sides of the tank.

The water in the tank was throughout clearer than in 1939 and was so on the day it was put in. Suitable flagellates were present whenever the water was examined, but on July 15 and August 9 the population appeared sparser than usual. On each of these dates two and a half to three litres of a thick culture of Flagellate "I" was poured in. This culture had been prepared—in readiness should it be needed—from a sample culture sent from Port Erin, where the species had proved to be excellent food for oyster larvae (Bruce *et al.*, 1940). Dr Bruce and Dr Parke kindly forwarded this culture at short notice. It is possible to question the value of these additions, but as data for a satisfactory discussion are lacking it can only be remarked that they possibly ensured the presence of a specially good food, in greater or lesser amount, depending on the extent to which multiplication of the added species took place.

Throughout the rearing the *p*H was not known to rise above 8·7 (corrected). Whenever a tendency to increased alkalinity was manifest enrichment was discontinued for a time. Temperatures were not taken regularly.

The first spat settled sometime not later than the first week in July and settlement continued into August. Early in the latter month, before the spat were able to settle too thickly, some of the suspended tiles were removed to tank B. Here they were given daily changes of water from the estuary. About the same time the breeding tank was topped with sea water from the estuary to make up for evaporation loss and leakage. It was emptied on August 15, when the tiles remaining in it were removed to tank C, there to receive daily changes of tidal water. Examination of the tiles during removal amply confirmed the findings of Cole & Jones (1939) that the larvae tend to settle high and in the darker places. The suspended tiles were well covered with spat, but the largest spat, up to 2 cm. in diameter, were on the flat tiles on the bottom. The better the under sides of these latter had been shielded from the light, the more thickly were they covered with spat, although they did not generally bear as many as did the suspended tiles. Illumination of the under surfaces of some of the flat tiles would be assisted by light reflected from the white oyster shells scattered over the bottom. There were algal growths on the under sides of the tiles with fewest spat. Very few spat settled on the oyster shells lying on the bottom: there were rather more on the shells hung in net bags. In future, after this experience, oyster shells will not be scattered over the tank bottom and every effort will be made to hang the main collectors close to the surface and to keep their under sides well shaded.

At the present time (mid-October 1940) large spat are 3 cm. in diameter with occasional ones as big as 3·5 cm. Some have already been chipped off the tiles and placed in wire cages in the estuary. The majority are, of course, smaller than this; 1–2 cm. across are very common, but many are still only 1 or 2 mm. They will remain on the tiles over the winter, most of them in tank C, but a few bundles have been hung on poles well above the mud near low-water mark.

A few spat have died, but the proportion is insignificant. We believe that this year's spat are more numerous, and on the whole larger at corresponding dates, than last year's.

It is reasonable to expect that the rearing of oysters on the River Yealm will become an annual routine. From a commercial point of view this will considerably enhance the value of the fishery by reducing, perhaps eliminating, the necessity of importing foreign brood; while for the immediate future it will overcome, partially at any rate, difficulties caused by the present impossibility of obtaining brood oysters from Brittany.

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NOTES ON BRITISH AND NORWEGIAN HYDROIDS AND MEDUSAE

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(Text-figs. 1–7)

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The notes collected in this paper are a continuation of earlier observations on British and Norwegian hydroids and their medusae. The observations were made at the Biological Stations of Herdla (Bergen), Millport and Plymouth.

Two of the hydroids described below, *Dipurena ophiogaster* Haeckel and *Tiaropsis multicirrata* (M. Sars), were previously unknown, although their medusae have been known for a long time. The earlier description of the medusa of the hydroid *Trichydra pudica* by Wright (1863) is confirmed and asexual budding in the hydroid *Coryne tubulosa* (M. Sars) is described. Miss Maude J. Delap has kindly allowed me to publish her sketches of the rare hydroid *Tricyclusa singularis* (Schulze).

I am much indebted to Dr Stanley Kemp, F.R.S., for many facilities and much encouragement. I also wish to thank Prof. August Brinkmann and Mr Richard Elmhirst for facilities at Bergen and Millport respectively. The work at Millport was carried out while I was holding a grant from the Royal Society.

ASEXUAL BUDDING IN THE HYDROID *CORYNE TUBULOSA* (M. SARS, 1835)

A large colony of *Coryne tubulosa* (M. Sars, 1835), better known as *Syncoryne sarsi* (Loven, 1836), was found growing in a plunger jar kept by Amanuensis D. Rustad at the Biological Station, Herdla, in September 1937. Mr Rustad kindly allowed me to examine the hydroid, which was creeping over the greater part of the bottom of a plunger jar. The colony was of the creeping form (Fig. 1 *a*), the stems usually bearing single hydranths, many with medusa buds. Some medusae had been liberated and were identified as young specimens of *Coryne tubulosa*. The most interesting feature of the contents of the jar, however, were "free hydranths" carrying two whorls of tentacles and sometimes also one or two medusa buds (Fig. 1 *b*, *c*).

A close examination of the hydroid on the bottom of the jar showed that many of these "free hydranths" were developing from the body of the fixed hydranths below the most proximal tentacles. These hydranth buds when liberated showed a striking similarity to the pelagic hydranths of *Margelopsis* (Fig. 1 *c*).

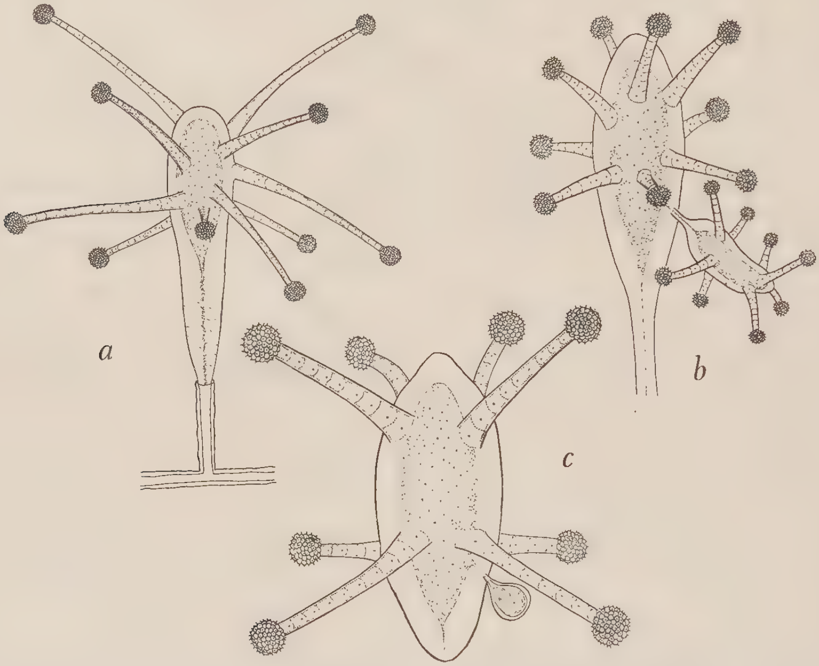

Fig. 1. *Coryne tubulosa*. *a*, Single hydranth from the colony brought back alive to Plymouth from Herdla; drawn 22. iv. 38. *b*, Single hydranth with fully developed bud, drawn from material fixed in formalin. *c*, Newly liberated hydranth bud with a young medusa bud, Herdla, 6. ix. 37.

There was never more than one bud on any hydranth and several buds became free from the parent hydranths during the time they were being examined. Some buds also carried medusa buds. The hydranth buds at liberation were 0·25–0·35 mm. long and 0·15 mm. in diameter. They carried two whorls of four capitate tentacles each, and in some there were one or two medusa buds borne on very short stalks immediately posterior to the proximal whorl of tentacles. The tentacles of the buds were about 0·2 mm. long with capitate terminal knobs 0·05 mm. in diameter.

Budding from the hydranth in this species has been observed by Hartlaub (1916). He figures a bud (p. 103, Fig. 22) similar to those found at Herdla.

Hartlaub also gives many other figures of budding in the same colony but these do not seem to be figures of normal polyps and I am inclined to regard many of them as abnormalities. During the first week of September at Herdla I found one of these "free hydranths" in a plankton haul taken close to the Biological Station. This method of budding "free hydranths" is probably a common feature in the life history of this hydroid when there is an abundant food supply available. A portion of this colony was brought back alive to Plymouth from Herdla, and although it was kept for many months, no further budding of daughter polyps was observed. A few medusae were, however, developed and liberated in May 1938.

THE HYDROID OF *DIPURENA OPHIOGASTER* HAECKEL

The hydroid of the medusa *Dipurena ophiogaster* Haeckel was found on the stipe of *Himanthallia loreata* by Miss M. J. Delap at Valentia Island in May 1904. Miss Delap sent the hydroid and the medusae reared from it to the late Mr E. T. Browne. This hydroid, of which there is no published description, is described here from a re-examination of the original colony found by Miss Delap.

The colony was small, consisting of upright stems arising from a creeping stolon and with a total height of about 4 mm. The stems bearing the hydranths arise from creeping stolons, 0·1–0·12 mm. in diameter. Both stems and stolons were covered by a thin perisarc without annulations. In well-developed stems the hydrocaulus reached a height of 2 mm., while in young polyp stems no perisarc was visible. The stems each carried a single hydranth, but short stolons were frequently given off a little distance from the point of origin of the upright stems (Fig. 2*a*). The production of these stolons on the stems was a characteristic feature of the colony.

Mature hydranths with medusa buds were 1–2 mm. long with a diameter of 0·2–0·35 mm. (Fig. 2*b*,*c*). There were two kinds of tentacles, capitate and filiform, present in most hydranths. The capitate tentacles, 10–18 in number, were scattered over the anterior two-thirds of the body; they had a tendency to be arranged in whorls. There was always an oral whorl of four tentacles around the mouth, the remainder being scattered. The capitate heads of the tentacles were 0·07–0·12 mm. in diameter and frequently decreased in size posteriorly.

The filiform tentacles were situated posterior to the capitate tentacles and varied in number in different polyps. There were never more than four on any hydranth (Fig. 2*b*), and frequently only one or two were present. In some hydranths they were absent and in others they formed part of a proximal whorl of capitate tentacles. A closer examination showed that these filiform tentacles always carried a number of nematocysts at the tip, and that transitional stages between capitate and filiform tentacles could be found in one whorl. In this species at least they may be regarded as reduced capitate tentacles (Fig. 2*c*).

Medusa buds were borne singly or in a cluster of two to four buds on the body of the hydranth a little anterior to the posterior whorl of "filiform" tentacles (Fig. 2*b*, *c*). Usually there was only one cluster but sometimes there were two. The young buds were sessile, but as they developed to full size they became stalked with a thin stalk up to 0·2 mm. in length. The largest medusa bud was 0·5 mm. long with a diameter of 0·45 mm. In the largest buds the manubrium, and the four tentacles with their ocelli, could be clearly seen through the thin perisarc covering the bud.

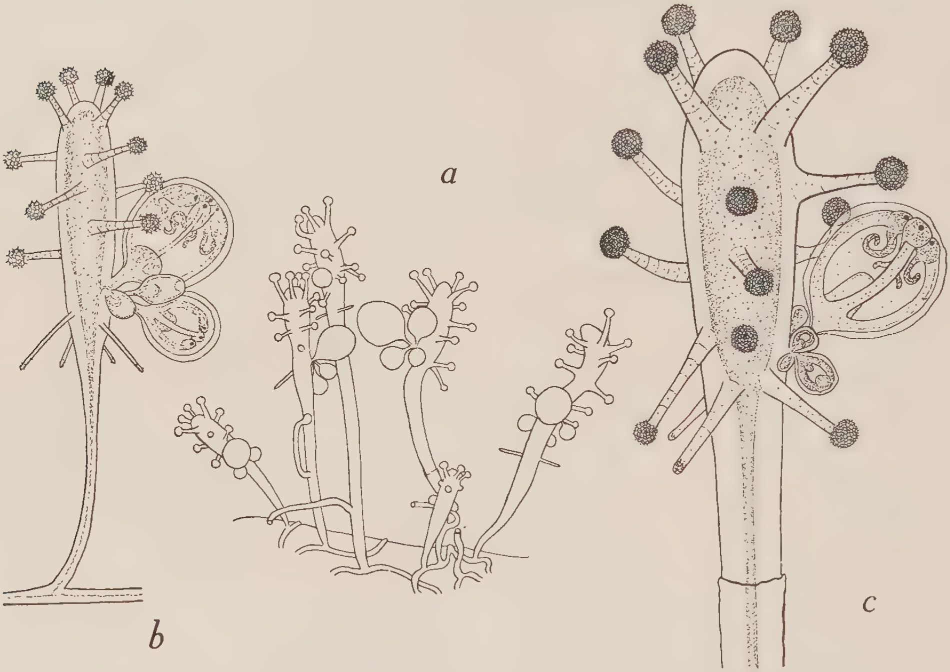

Fig. 2. *Dipurena ophiogaster*. *a*, General appearance of a portion of the colony, drawn from material preserved in formalin. *b*, Single polyp with medusa buds, redrawn from a pencil sketch of a living polyp by Miss M. J. Delap. *c*, Single hydranth, from material preserved in formalin.

The medusae reared from this colony by Miss Delap have been identified by Mr F. S. Russell as *Dipurena ophiogaster* Haeckel. This hydroid bears a superficial resemblance to *Stauridiosarsia* [= *Stauridium*] *producta* (Wright), but in the present hydroid the capitate tentacles are more scattered, there are only four oral tentacles, and the so-called filiform tentacles are really imperfectly developed capitate tentacles. The hydroid of *Dipurena ophiogaster* is also distinct from that of *D. halterata* (Forbes), which has more capitate tentacles, no filiform tentacles, and develops medusa buds singly (Rees, 1939*b*). Although the two hydroids which give rise to *Dipurena* medusae are well marked, the differences do not justify the allocation of the species to separate genera.

THE HYDROID AND FREE HYDRANTH OF *TRICYCLUSA SINGULARIS* (SCHULZE, 1876)

The following account of a rare and little known hydroid is based on figures and notes made by Miss Maud J. Delap. Miss Delap has kindly permitted me to publish an account of this hydroid, which I have identified with *Tricyclusa singularis* (Schulze, 1876) (= *Margelopsis stylostoma* Hartlaub, 1903, *syn.nov.*).

The Hydroid. Six specimens with buds were found on a piece of *Zostera* at Reenagiveen Point, Valentia Island, Co. Kerry, on July 19, 1909, and these were kept under observation by Miss Delap until July 30, 1909. The hydroid was attached to the *Zostera* by a short cylindrical stalk. The hydranth itself was more or less flask-shaped, with three whorls of capitate tentacles. Between the middle and oral whorl the body narrows to form a slender neck. The oral tentacles, which are terminal, are four in number, each with a terminal knob of nematocysts. There were six tentacles in the middle whorl. These and the proximal whorl carried two groups of nematocysts on their distal half in addition to the terminal knob. The proximal whorl, situated near the posterior end of the body, had twelve tentacles, disposed in two closely approximated whorls of six tentacles pointing upwards and six tentacles pointing downwards.

In one polyp the hydranth was cut off and the stem grew a new one. Some of the hydranths carried pink or pinkish yellow buds at the posterior end of the body (Fig. 3*a*). These developed into young hydranths which were budded off (Fig. 3*b*).

The young hydranths budded off in this way were identical, and may be regarded as synonymous, with *Margelopsis stylostoma* Hartlaub, 1903. Miss Delap states that "the young when liberated have fewer nematocysts and the tentacles appear longer than in the adult: they use their tentacles to crawl about with." A young specimen after liberation was 1 mm. long and the tentacles were 1·5 mm. long; in another specimen the tentacles were 0·5 mm. long.

The fixed hydroid differs in one respect from the description given by Schulze. There are fewer tentacles in the proximal whorl of the specimen figured by Schulze than in the present species, but their number probably varies with age and I have therefore referred this hydroid to *Tricyclusa singularis*.

SYSTEMATIC DISCUSSION ON THE MARGELOPSIDAE

The peculiar hydroid described above was first described by Schulze (1876) as *Tiarella singularis*. The name *Tiarella*, however, was preoccupied and Stechow (1919) has given it the new generic name *Tricyclusa*. The hydroid is of an unusual type, which is intermediate between the Tubulariidae and the Corynidae. Its remarkable power of budding young hydranths from the body, just behind the most proximal whorl of tentacles, was noticed by Schulze. He also observed gonophores developing between the middle and lower whorl of tentacles.

In 1903 Hartlaub published a description of a "schwimmender" hydroid, *Margelopsis stylostoma*, which he referred to his genus *Margelopsis*, of which *M. haeckeli* is the genotype. This latter species is a planktonic hydroid which superficially resembles *M. stylostoma*. Hartlaub drew attention to the marked similarity between his *M. stylostoma* and the hydranth of *Tricyclusa singularis*, and expressed the opinion that they might prove to be the same species. Hartlaub also indicated that the medusa *Margelopsis hartlaubi* Browne, 1903, might be the medusa of *Tricyclusa singularis*. The observations made by Miss Delap show that *Margelopsis stylostoma* comes from the fixed hydroid, *Tricyclusa singularis*.

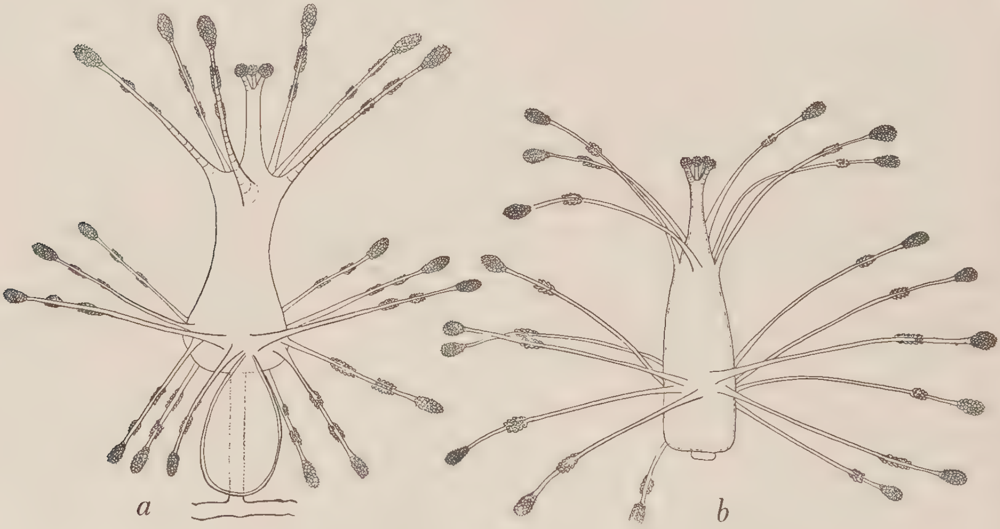

Fig. 3. *Tricyclusa singularis*. *a*, Fixed hydroid. *b*, Free hydranth. From tracings of original pencil drawings by Miss M. J. Delap.

Mayer (1910) erected a new subfamily of the Codonidae, the Margelopsinae, to take *Margelopsis* and *Pelagohydra* Dendy, 1902. The free hydroids of *Margelopsis haeckeli* and *Tricyclusa singularis* differ in several respects. There are only two whorls of tentacles in *Margelopsis haeckeli*, whereas *Tricyclusa singularis* has a third oral whorl; in the latter all the tentacles are capitate, but they are filiform in the former. These differences have evidently been considered by Stechow (1923), who, in his tables (pp. 37, 49), separates *Margelopsis* (i.e. *M. haeckeli*) from *Tricyclusa* in different families. He places *Margelopsis haeckeli* and *M. gibbesi* (McCrady) in the Tubulariidae in the subfamily Pelagohydrinae, of which *Pelagohydra* is the type; *Tricyclusa* he refers to the Corynidae. The separation of *Tricyclusa* from *Margelopsis* and the removal of the former to the Corynidae appears justifiable.

Uchida (1927) does not appear to have been aware of Stechow's suggestion and placed *Margelopsis*, *Hypolytus* Murbach, 1899, *Pelagohydra* and *Climacocodon* Uchida, 1924, in his family Margelopsidae. This arrangement appears to be satisfactory if we exclude *Tricyclusa* and *Hypolytus*. I have already indicated (Rees, 1938, p. 29) that *Hypolytus* clearly belongs to the Corymorphinae, if not to the genus *Corymorpha* itself. This species, according to Murbach, is capable of a certain amount of movement, but so are other species of *Corymorpha* (unpublished observations on *C. aurata* and *C. nutans*).

The medusae of *Margelopsis*, *Pelagohydra* and *Climacocodon* are all of the same type and are quite distinct from those of the Tubulariidae and Corynidae and justify the erection of a separate family. As Uchida (1924) has indicated, *Pelagohydra* is probably the only one of these genera that may be regarded as truly pelagic, and it has yet to be shown that the other forms have no fixed hydroid stage in addition to their free hydranths. Dendy has shown that in *Pelogohydra mirabilis* the proximal portion of the hydranth is modified to form a float, whereas a float is either poorly developed* or absent in *Margelopsis* and *Climacocodon*. There are other differences, such as the number and disposition of the tentacles, which indicate that *Pelagohydra* is not so closely related to the other two genera as they are to each other. On the other hand, the structure of the medusa in *Pelagohydra*, which is of the *Margelopsis* type, is proof that these forms are related.

I propose therefore to recognize two subfamilies, the Margelopsinae and the Pelagohydrinae, within the Margelopsidae.

The Margelopsinae may be distinguished by the possession of whorls of tentacles and by the absence of a distinct float. It contains the genera *Margelopsis* and *Climacocodon*.

The Pelagohydrinae may be distinguished by the scattered position of the tentacles all over the body of the hydranth, and by the modification of the proximal portion of the body to form a float. Sole genus: *Pelagohydra* Dendy, 1902.

THE MEDUSA OF *TRICHYDRA PUDICA* WRIGHT

Two well-developed colonies of the hydroid *Trichydra pudica* Wright, 1858, were found on two clinkers trawled off Fairlie Buoy in the Clyde Sea Area on April 11, 1940. This is the first record of the hydroid from this area. Previously it has been reported from the Firth of Forth by Wright (1858), from the English Channel (*Plymouth Marine Fauna*, 1931) and from Valentia Harbour, Co. Kerry (personal communication from Miss M. J. Delap). The hydroid which van Beneden (1866) described as *Eudendrium pudicum* is certainly not the present species (Rees, 1938).

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* Leloup (1929) has shown that in *M. haeckeli* the endodermal cells are vacuolated and that the structure of the short stalk of the hydranth resembles early stages in the formation of the float in siphonophores.

Wright (1863) described a medusa which he found in a vessel containing *Trichydra* and which he thought might be its medusa, but he could detect no trace of gonophores on his hydroid colony. On two clinkers from Fairlie there was a heavy growth of this hydroid and no other species were present. Two days later two medusae were liberated. Although a careful daily examination until April 19 revealed no trace of developing or fully developed medusa buds, six more medusae appeared in the bowl during this period. These clinkers with their numerous tiny pockets afford an excellent hiding-place for such buds, which must have been very small. I do not doubt that these tiny medusae came from the *Trichydra*; they agree in structure with those described by Wright except for the presence in Wright's specimens of two or three rudiments of interradial bulbs. Their presence or absence may vary from colony to colony.

A brief redescription of both hydroid and medusa is given below.

The polyps are connected by a creeping filiform stolon which is covered by a thin perisarc and from this stolon the polyps arise at short intervals. At the base of the hydranth there is a collar-like perisarc into which the hydranth can partially contract. This collar varies in length from 0·15 to 0·35 mm. and has a diameter of 0·1–0·14 mm. When disturbed the hydranth can contract so that only the tips of the tentacles show beyond the edge of the perisarc. When expanded, however, the hydranth may reach a height of 1 mm. beyond the edge of the perisarc, and in this state the stem supporting the hypostome and the tentacles is very thin with a diameter of about 0·03 mm. (Fig. 4). Distally the stem broadens to give rise to a whorl of tentacles, and beyond the latter there is a distinct conical hypostome. The tentacles when expanded may reach a length of 1·2 mm.; they carry numerous irregularly disposed nematocysts.

The first medusae were liberated in the laboratory on April 13 and others on succeeding days.

The newly liberated medusae were very small, having a length of 0·4 mm. and a diameter of 0·3 mm. They were of a deep bell shape, higher than wide (Fig. 5). The jelly was uniformly thin and all over the bell there were numerous nematocysts. The velum was well developed. The stomach was short, tubular, 0·14–0·17 mm. in length, and was distinctly broadened at its base. It was difficult to distinguish the stomach proper from the beginnings of the radial canals. The four radial canals were distinct and moderately broad. There were four perradial tentacle bulbs each about 0·05 mm. in diameter. The four perradial tentacles were not observed expanded, but when contracted they had a length of 0·15 mm. The base of the stomach and the tentacle bulbs were yellowish brown or brownish in colour. The medusae did not grow appreciably and did not grow more tentacles during the time I was able to keep them.

This young medusa cannot be identified with certainty with any known medusa because of the lack of any distinctive characters. It is probably a

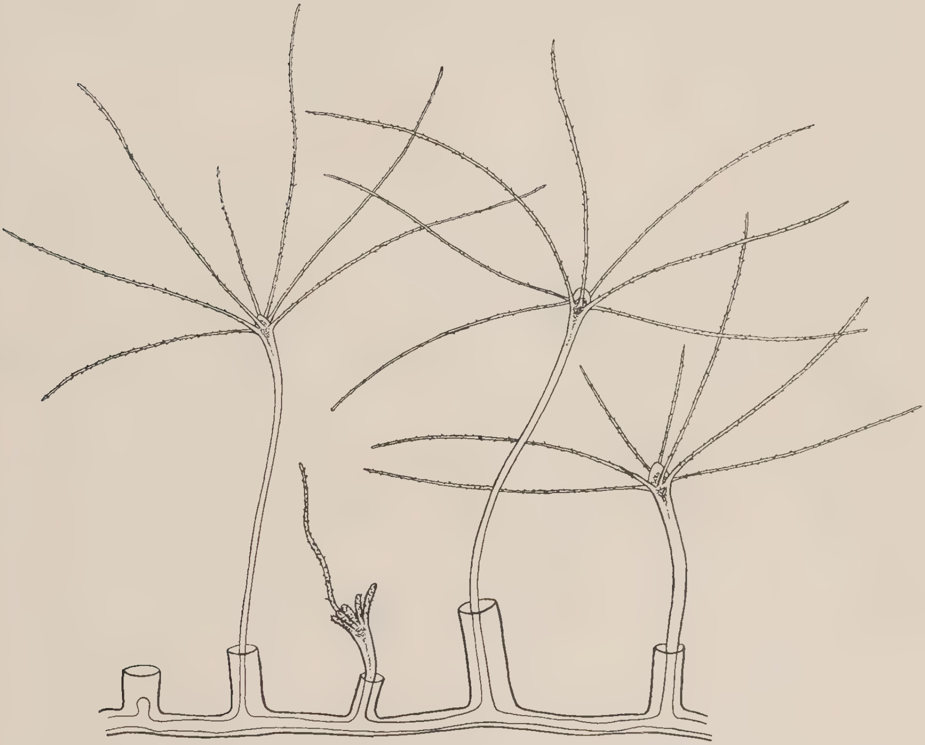

Fig. 4. *Trichydra pudica.* Four polyps from a colony; Millport, 12. iv. 40.

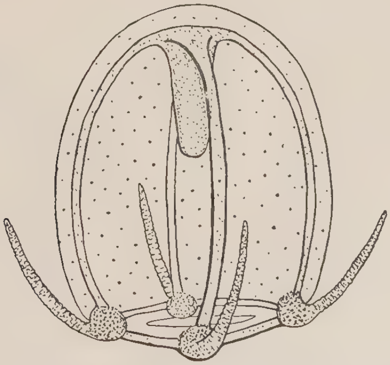

Fig. 5. *Trichydra pudica.* Newly liberated medusa; Millport, 15. iv. 40.

very early stage of a known medusa, and the form and colour of the tentacle bulbs suggest that it might possibly be an early stage of *Lizzia blondina* Forbes. The absence of mouth tentacles, however, does not support this view although their absence in such an early stage does not rule out the possibility that it might be this species.

THE HYDROID OF THE MEDUSA *TIAROPSIS MULTICIRRATA* (M. SARS, 1835)

The medusa *Tiaropsis multicirrata* was first described by Michael Sars (1835) as *Thaumantias multicirrata* and later by Louis Agassiz as *Tiaropsis diademata*. Agassiz mentions that the hydroid of this species is a *Campanularia* and that he had traced the whole life history. A. Agassiz (1865), however, stated that the hydroid had not been observed, so it appears that he was in some doubt about the earlier observation of his father. L. Agassiz (1849) definitely states that he had observed the planulae, "recently hatched from eggs, I have seen move slowly, then attach themselves to the solid bodies in the jars in which they were kept, and grow into a Campanularioid polypidom...".

Recent work on an operculate hydroid of the "*Campanulina*" type, which liberated a young specimen of *Tiaropsis multicirrata*, appears at first sight to contradict Agassiz's observations, but it must be remembered that at this time (1849) the genus *Campanularia* consisted of a heterogeneous group of hydroids including operculate and non-operculate forms. Apart from the vague record of the hydroid by Agassiz there is no description of the hydroid or of the newly liberated medusa and they are therefore described below.

The hydroid colony was found on an old *Buccinum* shell dredged off Keppel Pier, Millport, from a depth of about 5 fm. on April 18, 1940. No expanded hydranths were seen and of the few gonothecae present only one contained a medusa. This was liberated in the laboratory and was identified as *Tiaropsis multicirrata*.

Description of the Hydroid. The colony consisted of a large number of hydranths connected by creeping stolons running in all directions over the apical half of the shell. The stolons were 0·05 mm. in diameter and were not annulated. The hydranths were borne singly at short intervals on the creeping stolon and were supported on short imperfectly ringed stalks, which varied in length from 0·06 to 0·10 mm. The total height of the hydranths from the stolon to the tip of the operculum was never more than 1 mm. The hydrotheca itself varied from 0·38 to 0·80 mm. in length and in breadth from 0·1 to 0·13 mm. The perisarc of the hydrotheca was thin but firm and at its distal end became folded to form a distinct conical operculum with seven to eleven outer segments (Fig. 6). The operculum varied in height from 0·16 to 0·25 mm. When the operculum was closed the segments formed a somewhat blunt apex.

When found the colony was dying down after producing medusae and no hydrotheca contained a living polyp. Several gonothecae were present but all except one were empty. One gonotheca contained a single medusa which

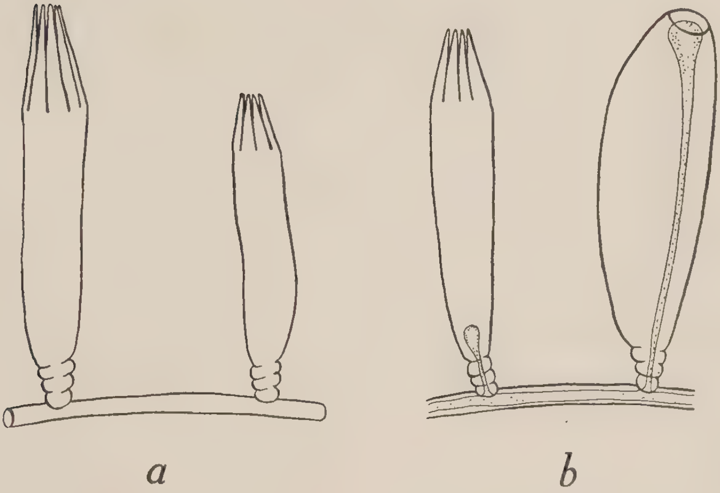

Fig. 6. *Tiaropsis multicirrata*. *a*, Two hydrothecae. *b*, One hydrotheca and a gonotheca; Millport, 19. iv. 40.

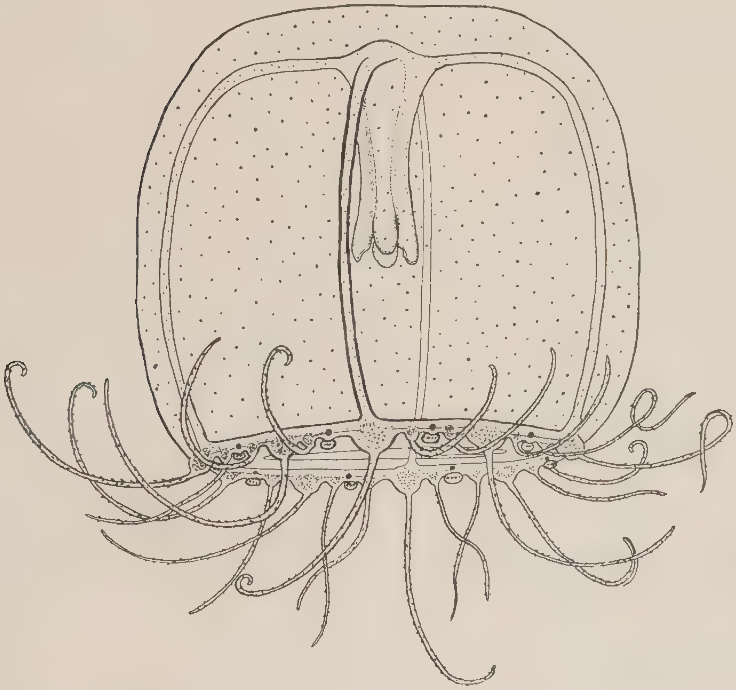

Fig. 7. *Tiaropsis multicirrata*. Newly liberated medusa; Millport, 19. iv. 40.

completely filled it. The gonothecae, like the hydrothecae, were supported on short imperfectly ringed stalks, and were large, elongate oval in shape, with a smooth surface. The mouth of the theca was circular, with a diameter much less than the maximum diameter of the theca itself (Fig. 6*b*).

Description of the Medusa. The newly liberated medusa had a deep bell-shaped umbrella with a thin jelly which was slightly thicker at the apex (Fig. 7). The surface of the umbrella was thickly covered by a large number of small nematocysts. There was a well-developed velum. There was a short quadrangular stomach extending to about half the height of the subumbrellar cavity. The mouth consisted of four well-developed perradial lobes, armed along their free margin by a single row of nematocysts. There were four moderately broad radial canals connecting with the ring canal. The marginal tentacles were many, twenty-three in number, and these were in different stages of development. The four perradial and the four interradial tentacles were equally well developed and when extended reached a length of 1·5 mm. There were also smaller tentacles, one on each side of the interradial tentacles and one on each side of the perradial tentacles. In one quadrant of this medusa (see Fig. 7) one tentacle, immediately adjacent to a perradial tentacle, was missing. Situated adradially and between these younger tentacles were eight prominent black ocelli and eight large oval marginal vesicles. The marginal vesicles measured 0·04 by 0·05 mm. and were adaxial in position on the umbrella margin. The vesicles contained from three to five spherical concretions.

The newly liberated medusa was 1·1 mm. high by 1·1 mm. in diameter. The larger tentacle bulbs were 0·1 mm. in diameter. They were pale brown in colour. The stomach was also faintly brownish in colour with the pigment concentrated more towards its base.

From its characters, especially the adradial position of the eight black ocelli and the eight marginal vesicles, there can be no doubt as to the identity of this young medusa, which can be referred to *Tiaropsis multicirrata*.

The hydroid described above is of the so-called "*Campanulina*" type, and when the shape of the gonotheca and the hydrotheca are taken into consideration, it appears morphologically to stand nearest to *Phialella quadrata* (Forbes, 1848). It has been shown by Rees (1939*a*) that a number of distinct medusa genera belonging to different families all possess "*Campanulina*" hydroids and thus the apparent morphological similarity between this hydroid and *Phialella quadrata* does not indicate that the species are related.

There are distinct differences between this hydroid and *P. quadrata*; the hydranths are all subsessile, the hydrothecae are longer and the gonothecae firmer than in *P. quadrata*.

Young specimens of *Tiaropsis multicirrata* with 25–35 tentacles appeared in plankton caught off Keppel Pier on March 5, 1940.

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ON THE HYDRORHIZA AND CLASPERS OF THE HYDROID *MYRIOTHELA COCKSI* (VIGURS)

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(Text-figs. 1–2)

THE HYDRORHIZA

The types of hydrorhiza shown by the northern hemisphere species of the hydroid *Myriothela* have been described by Bonnevie (1899) as follows:

I	II	III
Lamellary, expanded with chitinous perisarc	Tentacle-like, attaching filaments which grow from the base of the hydranth	Root-like point with fine attaching filaments, 10–20 mm. long
M. cocksi	*M. phrygia* *M. verrucosa*	*M. gigantea* *M. minuta* *M. mitra*

Among the southern hemisphere species the first type appears to be represented by *M. harrisoni* and the second by *M. australis*, *M. penola*, *M. capensis*, etc. Further details concerning *M. cocksi* were given by Hinks (1868), "the adherent base massive, of a dark horn colour, sending out a few tubular and root-like prolongations"; a small figure is also given, but it shows little detail of the form of the hydrorhiza where it is attached to the substratum. Allman (1875) describes and figures "short sucker-like processes of attachment" arising from the hydrorhiza which lies at right angles to the rest of the polyp.

During a recent examination of some southern hemisphere species of *Myriothela* particular attention has been paid to the manner of attachment of the hydrorhiza to the substratum in those species which show the second type of hydrorhiza (Manton, 1940). Since little is known of the structure and manner of attachment of the hydrorhiza of the first and third types referred to above, an examination of *M. cocksi* has been made with a view to remedying in part this deficiency. I am indebted to the Marine Biological Association for supplying me with well fixed material of *M. cocksi*, and to Dr L. E. R. Picken for carrying out chitin analyses.

The only other species which appears to show the first type of hydrorhiza mentioned above is the Australian *M. harrisoni*, where the cylindrical hydrorhiza "gives off slender rooting processes, expanded and truncated distally, for attachment to the substratum" (Briggs, 1928).

The lower portion of a young expanded specimen of *M. cocksi* is shown in

Fig. 1 *a*; the substratum has been removed from the hydroid. The hydrorhiza is roughly cylindrical in shape, but distorted to fit the irregularities of the substratum. The basal four-fifths of the hydrorhiza is encased in perisarc of a brown colour. The perisarc, unlike that of many hydroids, is everywhere adherent to the surface of the underlying ectoderm; it is fairly rigid, and its presence immobilizes this part of the hydroid. The perisarc is about 6μ thick. It stains red with Mallory's triple stain and black with iron haematoxylin. It is composed of true chitin. The dark colour probably indicates that the perisarc is hardened by impregnation, as is the cuticle of insects (Pryor, 1940 *a*, *b*).

Towards the blastostyle zone the perisarc becomes thinner, losing its staining reactions, and merges into the thin cuticle which covers the ectoderm of the hydranth. This cuticle is well shown in microphotographs of an allied species (Manton, 1940, pl. III, figs. 23 and 25). The zone covered by perisarc is shown by the mechanical tint in Fig. 1 *a*.

Below the blastostyle-bearing zone the axis of the hydrorhiza bears thirteen adhesive tentacles (*a.t.*) attached to the substratum and covered by perisarc. They are of various lengths up to 1·25 mm. One unattached tentacle arises above the zone covered by perisarc (*u.a.t.*), and is invested only by cuticle. Just above this lies the rudiment of another adhesive tentacle (*r.a.t.*), identified as such by sectioning.

The adhesion of the hydrorhiza to the substratum is effected only by the perisarc covering the flattened extremities of the adhesive tentacles (*a.d.*), and the perisarc here appears browner and thicker than elsewhere.

It is probable that growth of the hydrorhiza zone takes place orally, since young adhesive tentacles are found only at the oral unstiffened end of the hydrorhiza. In the region where the perisarc merges into the general cuticle the development of ectodermal musculature in the main axis is more complex than in other parts of the hydroid where these fibres form a regular longitudinal layer. Here the muscle processes lie in a variety of planes forming a network through the inner part of the ectoderm. It is possible that this musculature, besides controlling the position of the upper part of the hydroid, may be used to form local distortions in the flexible upper part of the hydrorhiza so as to produce contact between the young unstiffened adhesive tentacles and the substratum. The subsequent chitinization, thickening and hardening of the cuticle will then fix these distortions. The hydrorhiza figured here is young and simple, but the contorted hydrorhiza of gnarled appearance which is common on old specimens may have grown in this way.

In structure the unattached adhesive tentacle differs little from those of *M. penola* and *M. capensis* (Manton, 1940, text-figs. 6, 7). The tentacle is hollow, its enteric cavity opening directly into the main enteron. The ectoderm of the stem resembles that of other parts of the body and is provided with nematocysts. The terminal ectoderm forms a regular layer of darkly staining narrow cells, and nematocysts are almost absent. These cells do not contain

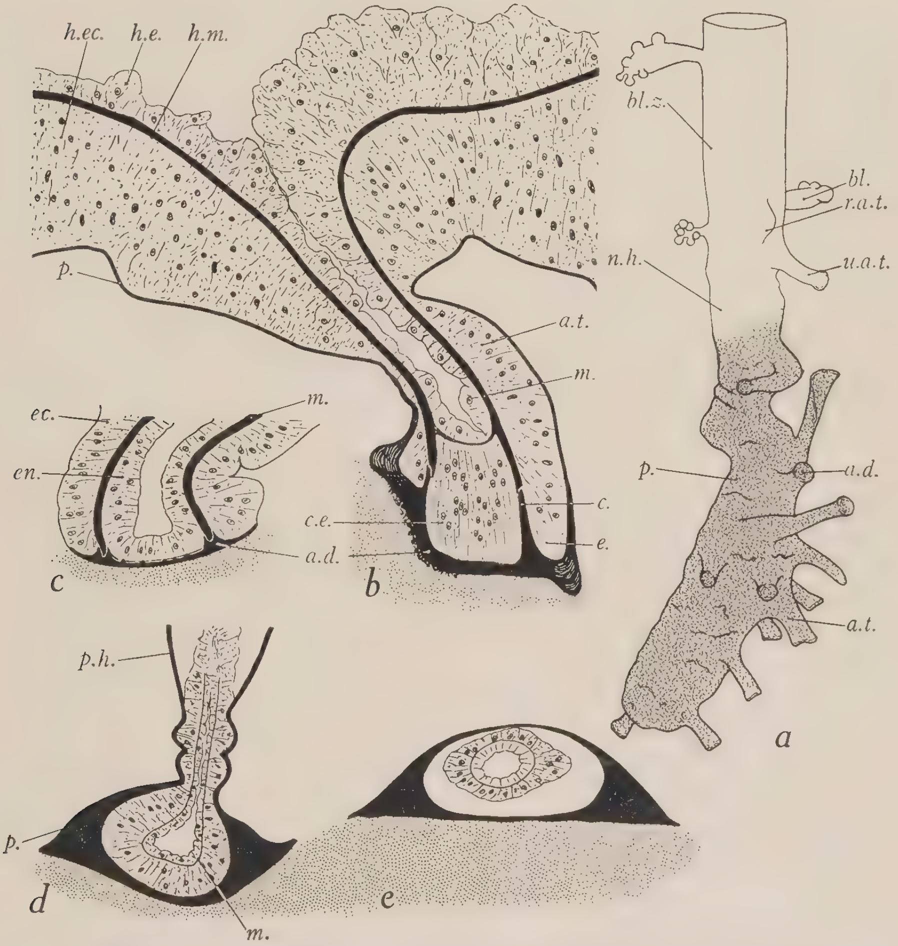

Fig. 1. *a*, the basal part of a young specimen of *M. cocksi*. The mechanical tint represents that part of the hydrorhiza which is covered by perisarc. *b*, sagittal section through an attached hydrorhiza tentacle and wall of the hydrorhiza of *M. cocksi*. The substratum is indicated by mechanical tint. *c*, sagittal section through an attached hydrorhiza tentacle of *M. capensis*. The substratum is indicated by mechanical tint. *d*, vertical section through the junction of hydrocaulus and hydrorhiza of *Obelia*. *e*, transverse section through a hydrorhiza stolon on *Obelia*.

a, × 12 approx.; *b–e*, × 110 approx.

a.d., adhesive disk of chitin attached to substratum. *a.t.*, adhesive tentacle of hydrorhiza. *bl.*, blastostyle. *bl.z.*, blastostyle-bearing zone of hydroid. *c.*, annular flange of chitin passing inwards from adhesive disk to unite with mesogloea. *c.e.*, central part of terminal ectoderm. *e.*, lateral part of terminal ectoderm. *ec.*, ectoderm. *en.*, endoderm. *h.e.*, hydrorhiza endoderm. *h.ec.*, hydrorhiza ectoderm. *h.m.*, hydrorhiza mesogloea. *m.*, mesogloea. *n.h.*, part of hydrorhiza not yet covered by perisarc. *p.*, perisarc. *p.h.*, perisarc of hydrocaulus. *r.a.t.*, rudiment of adhesive tentacle. *u.a.t.*, unattached adhesive tentacle.

the intracellular granules, which are present in the two above-named species, and are there destined to form the chitinoid layer. The mesogloea is thick along the stem of the adhesive tentacle, but becomes very thin distally, and here appears to be perforated in places.

The structure of an attached adhesive tentacle is shown in Fig. 1 *b*. The perisarc which covers the axis of the hydrorhiza and the stem of the tentacle is greatly thickened against the substratum. In this particular tentacle the terminal attachment fits into a hollow in the substratum and is humped, but most adhesive disks are flat. The perisarc is particularly thick round the rim of the disk. Just within this rim a deep annular flange of chitin projects into the terminal ectoderm, dividing the latter into a central (*c.e.*) and a lateral (*e.*) part. The mesogloea layer is prolonged into a thick rim projecting into the ectoderm to meet the chitinoid flange, as shown in the sagittal section figured, and the two structures unite. The adherent disk of chitin at the end of the tentacle is thus linked to the mesogloea. The junction between mesogloea and chitin is clearly seen after Mallory's triple stain, as the former stains blue and the latter from red to orange.

The perisarc shows no obvious structure other than a slight stratification parallel with the surface, and in sections it tends to split into upper and lower layers which appear to be identical. The chitin covering the tip of an adherent tentacle shows some slight colour difference from the rest. After Mallory's triple stain the deeper parts become orange red, and the superficial layers which have been torn from the substratum appear a purplish red. The process of adhesion by the tip of a tentacle has not been seen, but presumably this takes place at the time when the cuticle covering an unattached tentacle becomes thickened and hardened.

There appears to be much in common between the type of hydrorhiza shown by *M. cocksi* and that shown by *M. phrygia*, *M. capensis*, etc., a similarity not indicated by the literature. The second type, seen in *M. capensis* and *M. penola*, has been described by Manton (1940). Both types of hydrorhiza adhere to the substratum by the tips of tentacular structures, the adhesion taking place by means of chitinoid material which is also attached to the mesogloea. A sagittal section of an attached adhesive tentacle of *M. capensis* is shown in Fig. 1 *c*. In species showing this type of hydrorhiza a perisarc is usually described as absent, but it is in fact reduced to the small disk capping the bare adhesive tentacle (Fig. 1 *c*, *a.d.*). The naked hydrorhiza of such species is often very short, so that the adhesive tentacles tend to arise close together from the base of the hydranth, instead of at intervals from an elongated axis.

The general perisarc of *M. cocksi* must give some support to the points of adhesion with the substratum, and with the absence of the investing perisarc from species showing the second type of hydrorhiza a stronger attachment of the chitin to the mesogloea is seen. The mode of union between chitin and mesogloea described for *M. cocksi* (Fig. 1 *b*) is clearly repeated by *M. capensis*

(Fig. 1 *c*). The annular flange of chitin (Fig. 1 *b*, *c*.) in *M. cocksi* does not project inwards so far in *M. capensis*. The central portion of ectoderm (*c.e.*), which remains intact in *M. cocksi*, has disappeared in *M. capensis*, these cells breaking down during the formation of the chitinoid disk and leaving the latter closely adherent to the mesogloea right across the tip of the tentacle (Fig. 1 *c*). In *M. penola*, a giant species 850 mm. long, the large adhesive tentacles show a more extensive dove-tailing of the chitinous material into the mesogloea, a feature correlated with the greater size and greater strength needed per unit area of attaching surface.

It has been shown (Manton, 1940) that the chitinoid disks of *M. capensis* and *M. penola* are formed from granules of intracellular material situated in the terminal ectodermal cells, and the bases of these cells in *M. penola* extensively perforate the mesogloea. With the breakdown of these cells and the fusion of their granules chitinoid disks are formed which are thus anchored into the mesogloea. This method of formation of a chitinoid layer is not seen in *M. cocksi*, where intracellular granules are absent and the epithelium persists after chitinization of the cuticle. It is probable that the very unusual method shown by *M. capensis* and *M. penola* is associated with the extreme reduction in the extent of the perisarc, and the necessity for the remaining disk to be correspondingly strongly united with the soft parts of the hydroid. This method of chitin formation may be expected to occur in other species showing this type of hydrorhiza.

The hydrorhiza of many simple hydroids shows no elaboration for attachment such as is seen in *Myriothela*. *Obelia*, for example, possesses a very simple type of hydrorhiza consisting of extensive stolons lying over the substratum and attached to it along their adjacent surfaces. Sections of the hydrorhiza of *Obelia* are given for comparison with *Myriothela*. In Fig. 1 *d* a section is shown which passes longitudinally through the base of the hydrocaulus where it joins the hydrorhiza, and Fig. 1 *e* shows a transverse section of a stolon some distance from the hydrocaulus. Adhesion to the substratum takes place by the perisarc only, and no part of the coenosarc is involved. The perisarc is flattened against the substratum and is thickened along the outer edges of the stolon. The ectoderm may remain in contact with the perisarc, as in Fig. 1 *d*, but the coenosarc shrinks away from the perisarc in many regions situated remote from the hydrocaulus or from the growing tip of the stolon. The mesogloea (*m.*) is very thin and is unconnected with the perisarc (compare Fig. 1 *d* with 1 *b* and 1 *c*).

The perisarc of the hydrorhiza of *Obelia* is thick, but it is not stiff or hard as is that of *Myriothela*. It stains blue with Mallory's triple stain, and is composed of almost pure unhardened chitin. In these hydroids the pure chitin is flexible and pale in colour, just as is the unimpregnated unhardened cuticle in insects (Pryor, 1940*a*, *b*).

METHOD OF GROWTH OF THE HYDROID

It has been shown (Manton, 1940) that in *M. penola* increase in size of the polyp takes place from definite growth zones. The tentacle-bearing region elongates mainly from the oral end, and the blastostyle-bearing zone grows orally at the expense of the basal part of the tentacle-bearing zone. The same method of growth is shown by *M. cocksi*, young tentacles are formed round the mouth, and a young specimen shows immature blastostyles arising on the lower part of the tentacle-bearing zone before the tentacles are all absorbed. The hydrorhiza in both species grows in length at its oral end (see above).

THE CLASPERS

The "claspers" of *M. cocksi* have for long been regarded as remarkable or unique structures. Many new species of *Myriothela* have been described recently, but none shows this feature. The appearance and function of the claspers in *M. cocksi* in carrying the fertilized and developing ova has been described by Allman (1875), Benoit (1925), and others. The claspers are tentacle-like structures arising from or near the bases of the blastostyles, and the eggs are carried on their extremities, several claspers often adhering to one egg membrane.

A slight overlapping of the hydrorhiza and blastostyle-bearing zones has been described in *M. penola* and *M. phrygia*, where some of the adhesive tentacles of the hydrorhiza arise from or near the bases of a few of the proximal blastostyles (Manton, 1940). The adhesive tentacles in this position bear a striking superficial resemblance to the claspers of *M. cocksi*. Moreover, inspection shows that the method of adhesion of the claspers to the egg of *M. cocksi* differs little from that of a hydrorhiza tentacle to its substratum.

An unattached clasper in structure resembles a free hydrorhiza tentacle in that the ectodermal and endodermal epithelia are complete, the mesogloea at the tip is thinner, denser, and more darkly staining with Mallory's triple stain, and is here perforated by the bases of some of the ectodermal cells. The tip of a clasper first adheres to the egg membrane by the surface of its ectodermal cells (see Benoit, 1925, text-figs. 24–26), the ectodermal epithelium being intact. Further changes in the clasper are not described by Benoit, but he figures an older clasper with only one layer of cells at its tip (Benoit, 1925, text-figs. 27–28), a feature also shown by Korotneff (1888, pl. 2, fig. 10). A sagittal section through an attached clasper which is older still is shown here in Fig. 2. The mesogloea at the tip has thickened, particularly at the margin of the adhesion, and has united directly with the egg membrane. The ectoderm at the tip of the clasper has disappeared. The only difference between the adhesion of the clasper and that of a hydrorhiza tentacle of *M. capensis* or *M. penola* is the absence of a chitinous disk of adhesion in the former; in both a mesogloea attachment is made and the terminal ectoderm has disappeared (compare Figs. 1 *c*, 2).

Thus the similarity between (i) the position of the claspers of *M. cocksi* and some of the hydrorhiza tentacles of *M. penola* and *M. phrygia*, and (ii) the structure and method of adhesion of the claspers of *M. cocksi* and the hydrorhiza tentacles of other species of *Myriothela*, suggests that the claspers represent a specialized part of the hydrorhiza which has altered its function and now adheres to ova instead of to the substratum.

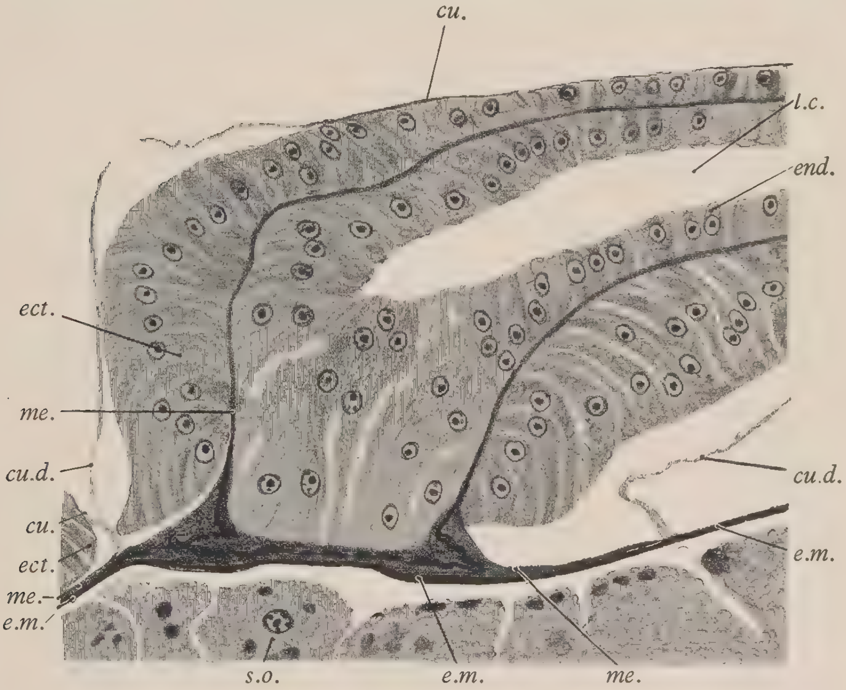

Fig. 2. Section passing sagittally through a clasper and part of the ovum to which it is attached in *M. cocksi*. The edge of a second clasper is seen on the left. The cuticle of the clasper is raised from the epithelium in places (*cu.d.*) owing to shrinkage of the tissues since death. The mesogloea of the clasper is clearly united with the egg membrane, the terminal ectoderm having disappeared.

cu., cuticle. *cu.d.*, cuticle detached from epithelium by shrinkage. *ect.*, ectoderm. *e.m.*, egg membrane. *end.*, endoderm. *l.c.*, endodermal lumen of clasper. *me.*, mesoglöea. *s.o.*, segmenting ovum.

SUMMARY

The hydrorhiza of *Myriothela cocksi* is described. It bears adhesive tentacles covered by perisarc, which adhere to the substratum by their flattened extremities.

The chitin forming a disk of adhesion is thick, and is attached to the mesogloea of the tentacle. The terminal ectoderm persists.

The hydrorhiza of *M. cocksi* and of species such as *M. capensis*, *M. penola*, and *M. phrygia*, etc., is built on a common plan. In the latter species the perisarc is reduced to adhesive disks more firmly attached to the mesogloea.

The method of growth of *M. cocksi* is recorded.

The structure and method of attachment of the claspers of *M. cocksi* to the egg membrane is described. The mesogloea at the tip of the clasper fuses with the egg membrane after the disappearance of the terminal ectoderm.

Evidence is given for the view that the claspers of *M. cocksi* represent a specialized part of the hydrorhiza.

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VERTEBRAL VARIATION IN TELEOSTEAN FISHES. II. THE HERRING (*CLUPEA HARENGUS* L.)

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(Text-figs. 1–4)

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INTRODUCTION

In October 1935, investigators from eleven countries represented on the International Council for the Exploration of the Sea met at Lowestoft to discuss the co-ordination of the results of herring research (Cons. Int. 1936, p. 7). The first four of the eight resolutions made at this meeting may be quoted as an introduction to the subject-matter of the present paper:

> (1) It is recommended that any group of herring spawning in a given area at a given season from year to year should be defined as the natural "Biological Group". Morphological characters should be used as a practical aid to the identification of these biological groups outside the area and season of their spawning.
>
> (2) It is recommended that work on biological groups and other local and seasonal populations should be continued in order to establish and test the continuity of the morphological characters of the biological groups, so that the members of the group and possibly the products of their spawning may be traced as they occur in the fisheries in later life. This will afford research workers data as to the effect of fishing on particular shoals and material on which to base predictions.

(3) As the biological groups of herrings are in many cases distinguishable by morphological characters, it is recommended that vertebral counts of selected material should be continued, additional characters such as the number of keeled scales being also studied when necessary.

(4) It is recommended that a co-operative study be undertaken on the practicability of utilizing a count of separate portions of the vertebral series in such a way as to draw distinctions between morphologically distinguishable groups with a minimum of labour and material. Mr Ford accepted an invitation to prepare a scheme. Professor R. A. Fisher consented to act in an advisory capacity.

My personal association with resolution 4 was the outcome of my statement at the Lowestoft meeting that there is a well-differentiated group of from four to eight vertebrae at the tail-end of the herring backbone which can be counted with precision; and that the use of this much shorter count as an alternative to the more familiar "total number of vertebrae" in population analyses might perhaps give equally satisfactory results. But, since then, a study of vertebral variation among teleostean fishes in general (Ford, 1937) has revealed an embarrassing lack of information of the kind required for the effective use of vertebral characters in population studies. In the pages which follow, therefore, the main effort has been to draw attention to the changes in form along the length of the herring backbone, and to illustrate by actual data the extent of individual variation which may be expected when working with statistical samples. At the same time, the ultimate purpose of the work, viz. the utilization of counts of separate portions of the vertebral series in such a way as to draw distinctions between morphologically distinguishable groups of herrings (vide Lowestoft resolution 4), has been borne in mind throughout.

PRELIMINARY CONSIDERATIONS

There is no mistaking the backbone of a herring for that of any other fish. For despite the numerous points of agreement in anatomy with the backbones of allied species and genera within the great order Isospondyli and, indeed, with those of other orders as well, the attributes of *Clupea harengus* are discernible not only in the backbone as a composite whole, but in each and every vertebral component throughout its length.

There is at least the possibility that the species *C. harengus* exists in nature in a number of distinct subspecific forms, each with its own bodily characteristics, geographical distribution and established habits of migration, feeding and spawning. If this is so, it might be expected that the backbone, which so plainly reveals specific identity, would also afford clues to subspecific identity. That is to say, one would be able to determine from the backbone alone the particular subspecies to which any individual herring should be referred. And this could be done just as surely for a fish caught outside the area and season of spawning as for one taken from a spawning shoal.

On the other hand, the form of the herring backbone varies to a marked degree from individual to individual—so much so, that in practice it is simply a matter of routine to resolve any sample of, say, one hundred backbones into one hundred separate backbones, each visibly different in structure from all the rest. Hence, even if the species exists as a number of morphologically distinguishable subspecies, appreciable individual variation in backbone form must be expected in each of them.

Taking these facts into consideration, it becomes clear that a sharp distinction should be drawn between (*a*) a backbone character which, by itself, is definitely diagnostic of the species or subspecies, and (*b*) one which is primarily a measure of vertebral variation, and which only acquires significance as a clue to identity when it is taken in conjunction with corresponding data for other backbones. Characters of class (*a*) may be likened to "hallmarks", which at once establish identity in the individual and will only be seen in *C. harengus*, or in one particular subspecific form of *C. harengus*. It need hardly be said that the study of local populations of herrings and of their migrations would be rendered very much more straightforward if reliable characters of this kind could be determined. The matter has already received preliminary attention, with encouraging results, but it is as yet too early to publish a report. In the meantime, therefore, it is necessary to deal for the most part with backbone characters of class (*b*) which, in contrast with the positive anatomical attributes just referred to, are but statistical measurements. They are the raw data on vertebral variation of a particular kind among the particular group of individuals from which they have been derived. As such, they must be subjected to recognized statistical treatment if they are to yield the special information required. The technique is the routine one of assessing the amount of variation in statistical samples, and comparing the results. For this, counts along the vertebral series in accordance with distinct changes in form are obviously the most serviceable, and the ultimate preferment of one count over another will rest, first, on the degree of precision with which the count can be made, and secondly, on the amount of labour and material involved in its determination.

Seeing that a great deal of work has already been done regarding variation in the so-called "total number of vertebrae", it may be asked why it should be necessary at this late stage to discuss the use of alternative counts? In reply it will probably be sufficient to advance three points:

(1) Experience has shown that in the determination of the total count in each of, say, one hundred herrings, some doubt will arise as to the strict validity of the count in perhaps twenty individuals, because of some observed abnormality in structure (Ford, 1933).

(2) Backbones composed of the same number of vertebrae may nevertheless differ in other respects; conversely, those which differ in the total may yet have other anatomical features in common. Consider, for example, the simple division of the backbone into an anterior part (*a*) and a posterior part (*b*) in accordance with a change in form at the junction of the two parts. The same total of 56 vertebrae is arrived at when (*a*) is 50 and (*b*) is 6, as when

(*a*) is 51 and (*b*) is 5; alternatively, a total of 56 expressed as (50 + 6) differs from a total of 55 expressed as (49 + 6), although the value of (*b*) is the same in both.

(3) This point is suggested in part by the examples used above, namely, that in certain circumstances a much shorter count along the backbone may give as much information as the total count. Thus, the difference between a total count of 56 and a second of 55 may prove to be no more than a difference of 1 in the value of (*b*) which is 6 in the one backbone and 5 in the other, the value of (*a*) being 50 in both. When it is remembered that before any direct count along the backbone can be made, the parts to be counted must first be properly exposed to view, whether by X-ray, by dissection, by selective staining, or by preparing a whole skeleton, it will be realized that the shorter the length of backbone to be handled, the less the work of preparation and counting. Apart from this matter of practical economy in working, however, there is another sound reason for thinking that a different system of counting may sometimes yield more conclusive results. This has to do with the basic metamerism of the herring. Investigation has shown that in the anterior part of the body the basic metamerism remains to a large extent unimpaired, so that the vertebrae, ribs, myocommata and certain of the keeled scales along the mid-ventral line of the body are in step with one another. That is to say, a count of the keeled scales, provided that it begins and ends at the right place, is in effect a vertebral count. Details are given later on p. 168, and it will be sufficient here to say that such a count, being an expression of the fundamental constitution of the herring's bodily structure, is one of special interest in present considerations.

THE ANATOMY OF THE HERRING BACKBONE

The herring backbone has a characteristic form pattern in consequence of successive alterations in form from vertebra to vertebra along its length. Analysis shows that each of the vertebral components—the centrum, neural and haemal arches and their processes, the zygapophyses, etc.—changes in form from one vertebra to the next in its own appointed way, and that for the most part these form changes are evenly graded. At a number of points along the backbone, however, there is an abrupt change in the form of a component. Thus, at one point the form of the haemal arch will alter appreciably between adjacent vertebrae, while at a second point the form change will be seen in the neural arch, and so on. By determining the position of all such points along the backbone, the primary form pattern can be expressed in terms of a number of secondary patterns, each covering a well-differentiated group of vertebrae within which there is a distinctive gradation of form in at least one vertebral component. Moreover, it may be added that each of these secondary patterns is as characteristic of the herring as is the primary pattern.

Immediate interest centres in the number and serial position of the vertebrae over which the primary and secondary patterns are spread, since these vary from individual to individual within the range shown by the species. In Fig. 1 the left side of a backbone, consisting of a total of 56 vertebrae between the skull and the terminal urostylar segment, is marked off in accordance with changes of pattern. It so happens that in the particular backbone represented in the figure the right side agrees with the left in the details portrayed, but it is important to note that such complete agreement between the two sides is by no means usual. The known frequency with which bilateral

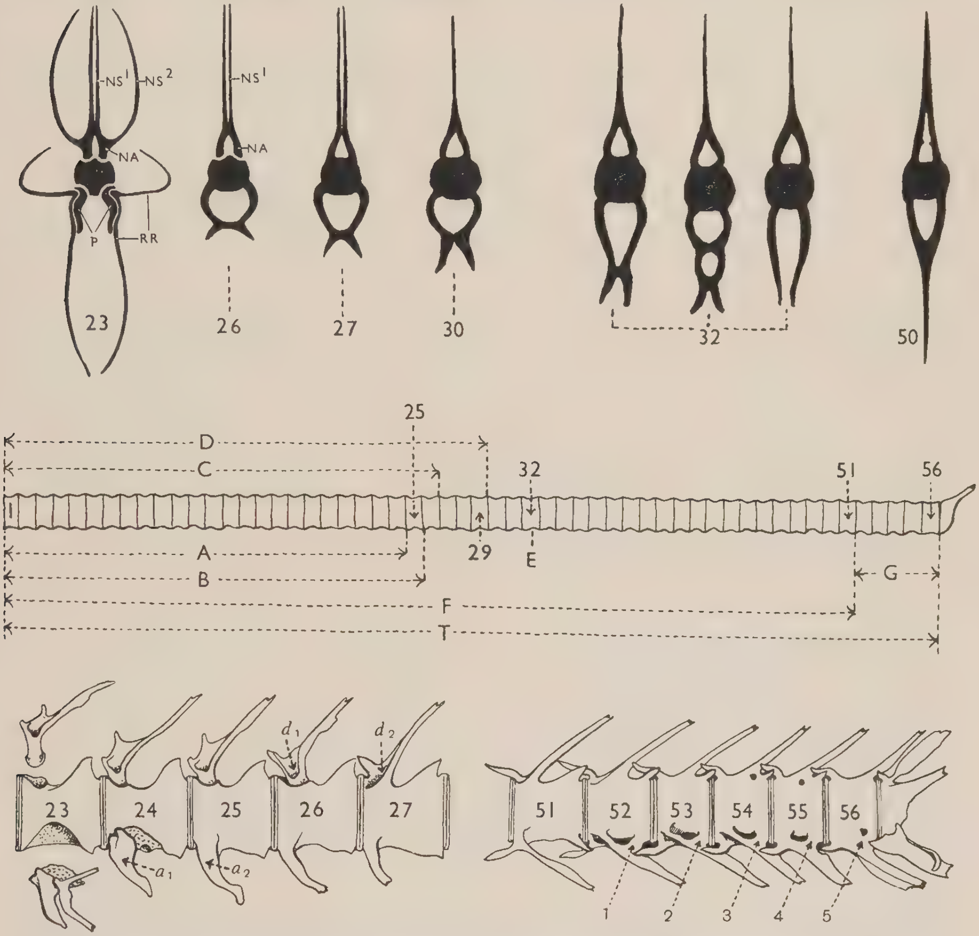

Fig. 1. Above: profiles of key vertebrae; three alternative forms of the 32nd are shown. Centre: diagrammatic outline of backbone viewed from left side. Below: outline of two sections of backbone viewed from left side.

The numbers represent serial numbers of the vertebrae: for particulars of *A*, *B*, *C*, *D*, *E*, *F*, *G* and *T*, see text, p. 156.

asymmetry occurs renders it necessary in practice to distinguish between counts along the left side and those along the right. Coming back to Fig. 1, and remembering that in this instance the two sides are in agreement, it is seen that the backbone is composed as follows:

Count denoted by				
Left side	Median line	Right side	Group of vertebrae counted	Last vertebra in count
A_l		A_r	Pre-caudal vertebrae with autogenous haemal arch	24
	B		Pre-caudal vertebrae with open haemal arch	25
C_l		C_r	Vertebrae with autogenous neural arch	26
	D		Vertebrae with duplicated neural spine	29
F_l		F_r	The "trunk" vertebrae, i.e. all the vertebrae except those at the tail-end in which the haemal spines are cross-tied to the centra	51
	T		All the vertebrae between the skull and the terminal urostylar segment	56

A further count E is not included in the above summary because it is not of the same category as those given. It marks the vertebra in which there is normally a sudden increase in the size of the haemal canal to provide housing for a blood vessel to the kidney. In the backbone figured this is seen in vert. 32. Reference should also be made here to the fact that the number of vertebrae in the "tail" group, although not specifically indicated in the summary, is given by $G_l = (T - F_l)$ for the left side, and $G_r = (T - F_r)$ for the right. Alternatively, of course, G_l and G_r can be directly determined as a separate count on the actual backbone.

Now it can be stated without hesitation that the above series of counts is a highly discriminating description of any herring backbone. One might examine hundreds of specimens before obtaining two which did not differ in at least one respect. It could hardly be otherwise, seeing that all the counts named are subject to variation from fish to fish, even among those of the same biological group as defined in Lowestoft resolution 1.

In the routine determination of counts in samples of fish, certain irregularities in vertebral form are likely to be discovered which affect one or more of the counts in some 20 % of a sample. The chief of these irregularities are:

(i) Fusions of adjacent vertebrae.

(ii) Duplication of the neural and haemal spines on the two vertebrae immediately preceding the urostylar element.

(iii) Irregularity in character E.

The occurrence of fused vertebrae (see Fig. 2 on p. 157) has been dealt with in an earlier paper (Ford & Bull, 1926). The fusion may occur at almost any point along the backbone, although more often at some points than at others. If these fusions are counted, not as single units but as the number of vertebrae apparently involved in the fusion, the total number of vertebrae is restored to the "normal".

Duplication of neural and haemal spines on the two vertebrae immediately preceding the urostylar element (see Fig. 2 on p. 157) has also been the subject of an earlier report (Ford, 1933). The counts of the vertebrae in the "tail" group of vertebrae, i.e. counts G_l and G_r, are particularly affected by this irregularity, and it is therefore advisable in statistical work to treat such cases in a class apart from the rest.

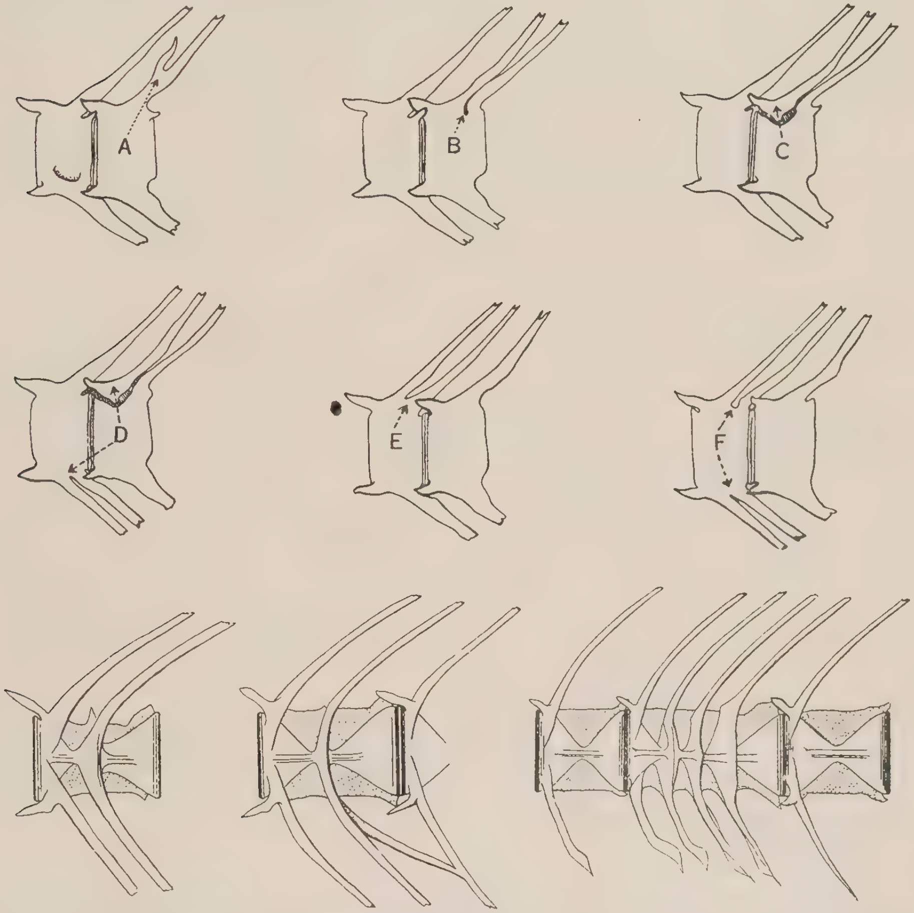

Fig. 2. Above: double-spined vertebrae (*A*, *B*, *C*, *D*, *E* and *F*) at the tail-end of herring, from Ford (1933). In each of the diagrams the two vertebrae represented are those immediately anterior to the terminal (urostylar) segment.

Below: abnormal vertebrae in the herring, from Ford & Bull (1926).

Normality in the secondary count *E* may be described simply as an obvious enlargement of the haemal canal to give easy passage for a blood vessel to the kidney. In some backbones, however, either the closed haemal canal is divided into separate upper and lower compartments, or the canal is open between a large bony loop formed by the haemal arches of the two sides of the vertebra (see Fig. 1).

Individual Variation in Vertebral Counts

The range of variation which may be expected in working through a sample of 100 herrings is satisfactorily indicated by actual data for a sample taken from a drift-net catch at Padstow in Cornwall in December 1935. All the counts were made on whole skeletons prepared by boiling and cleaning. Each count will be discussed in turn.

Counts A_l and A_r

The number of vertebrae with autogenous haemal arches varies as follows:

	No. of vertebrae				Total no. of backbones
	22	23	24	25	
A_l (left side)	12	57	29	2	100
A_r (right side)	11	58	29	2	100
Sum	23	115	58	4	A. mean = 23·215

At first sight it would appear from the above summary that there is little difference in the counts along the two sides of the backbone. Actually, however, the left side count differs from that of the right side in 41 backbones, as will be seen from the alternative summary:

	No. of vertebrae						
$\frac{A_l \text{ (left side count)}}{A_r \text{ (right side count)}}$	$\frac{22}{22}$	$\left(\frac{22}{23} \text{ or } \frac{23}{22}\right)$	$\frac{23}{23}$	$\left(\frac{23}{24} \text{ or } \frac{24}{23}\right)$	$\frac{24}{24}$	$\left(\frac{24}{25} \text{ or } \frac{25}{24}\right)$	
No. of backbones	4	15	39	22	16	4	= 100

Total of 59

It is seen that A_l is the same as A_r in 59 backbones, and although the most frequently occurring value for both A_l and A_r is 23, not more than 39 backbones give this count along both sides.

Count B

The number of vertebrae with open haemal arch varies as follows:

No. of vertebrae					Total no. of backbones	
22	23	24	25	26		
3	26	55	15	1	100	A. mean = 23·850

This is a well-known count which has often been made in past work. Objection has been raised to it on the ground that partial bridging across the haemal arches may occur in several vertebrae anterior to the one in which the complete closure is seen. Orton (1916, p. 80), for example, in recording the serial number of the first vertebra having a complete haemal arch, considered it advisable to state in addition the number of vertebrae having an incomplete but "well-developed" arch—and an arch was recorded as "well-developed" if the haemal processes were almost as large as those of the first complete arch and possessed even the smallest trace of an internal cross-piece. In his opinion, it is not improbable that in the living animal these incomplete but well-developed arches are closed by a cartilaginous cross-piece. Hence, he would seem to favour a statistical count of the number of vertebrae "with potentialities" for complete haemal arches, rather than a count which includes only those vertebrae in which the haemal arch is unquestionably closed by a bony bridge. Certainly, the matter is one which should be borne in mind by any investigator proposing to use the counts in statistical work.

Counts C_l and C_r

The count of the number of vertebrae with autogenous neural arches varies from fish to fish and from side to side. Dealing first with the separate counts C_l and C_r:

	No. of vertebrae					Total no. of backbones
	24	25	26	27	28	
C_l (left side)	1	11	49	33	6	100
C_r (right side)	—	12	42	39	7	100
Sum	1	23	91	72	13	A. mean = 26·365

As with the counts A_l and A_r, the extent of bilateral asymmetry is best indicated by an alternative summary of the data:

	No. of vertebrae							
$\frac{C_l \text{ (left side count)}}{C_r \text{ (right side count)}}$	$\frac{24}{25}$	$\frac{25}{25}$	$\left(\frac{25}{26} \text{ or } \frac{26}{25}\right)$	$\frac{26}{26}$	$\left(\frac{26}{27} \text{ or } \frac{27}{26}\right)$	$\frac{27}{27}$	$\left(\frac{27}{28} \text{ or } \frac{28}{27}\right)$	$\frac{28}{28}$
No. of backbones	1	5	12	28	23	21	7	3 = 100

Total of 57

The data show that C_l is the same as C_r in not more than 57 out of the total of 100 backbones. The most frequent value for both C_l and C_r is 26, yet only 28 backbones give this count along both sides.

Count D

The number of vertebrae in which the neural spine is duplicated shows the following variation:

No. of vertebrae				Total no. of backbones	
28	29	30	31		
5	32	55	8	100	A. mean = 29·660

Count E

As stated above, it is necessary in making this count to anticipate instances of irregularity in the form of the haemal arch concerned. In the present sample there are 11 backbones in which either the haemal canal is divided into upper and lower compartments, or the haemal arches form a large open loop. The variation in count E, shown by (*a*) the 89 normal backbones and (*b*) the 11 irregular ones, is shown below:

	No. of vertebrae				Total no. of backbones	
	31	32	33	34		
Normal	2	47	38	2	89	A. mean = 32·449
Irregular	2	7	2	—	11	A. mean = 32·000
Sum	4	54	40	2	100	A. mean = 32·400

Attention is drawn to the lower average value of the count in the irregular backbones. Although the number of such backbones in the present sample is much too small for statistical purposes, there is reason to believe that the lower average is not an entirely fortuitous result, since it has been observed in a number of other samples. One indication is that the irregular form of haemal arch is more common in backbones with a total of 55 vertebrae, excluding the urostyle, than in those with a total of 56, but, here again, the observation needs statistical confirmation.

Counts F_l and F_r

It will be seen from Fig. 1 on p. 155 that the backbone is divisible into the anterior and larger "trunk" group of vertebrae, followed by the posterior and much smaller group of "tail" vertebrae. Counting along the left side of the backbone, therefore, the total number of vertebrae (T) is the sum of the "trunk" count (F_l) and the "tail" count (G_l). Along the right side, (T) is the sum of (F_r) and (G_r). In the Padstow sample, only 85 backbones were "normal" in the sense that straightforward counts of "trunk", "tail" and "total" vertebrae could be made along both sides. In the remaining 15 this was not possible on account of some irregularity in vertebral form towards the posterior end of the backbone which interfered with the counts. For the normal backbones the values of F_l and F_r varied as follows:

	No. of vertebrae					Total no. of backbones
	48	49	50	51	52	
F_l (left side)	—	8	44	32	1	85
F_r (right side)	1	7	38	36	3	85
Sum	1	15	82	68	4	A. mean = 50·347

The degree of individual discrepancy between F_l and F_r is indicated below in the same manner as before.

$\frac{F_l}{F_r}$	$\frac{48}{49}$	$\frac{49}{49}$	$\left(\frac{49}{50} \text{ or } \frac{50}{49}\right)$	$\frac{50}{50}$	$\left(\frac{50}{51} \text{ or } \frac{51}{50}\right)$	$\frac{51}{51}$	$\frac{51}{52}$	$\frac{52}{52}$	
No. of backbones	1	6	2	36	8	29	2	1	=85

Total of 72

Thus, in 72 out of the total of 85 backbones, the left side count F_l is the same as the right side count F_r, which is a greater proportion than with the counts *A* and *C*.

Counts G_l and G_r

The first of the "tail" group is distinguished from the last of the "trunk" group by the fact that in the former the haemal spine is cross-tied to the centrum by a bony strut (see Fig. 1 on p. 155). As with counts *A*, *C* and *F*, there is some measure of bilateral asymmetry, so that it is necessary to distinguish between the left and right side counts G_l and G_r. In the 85 normal backbones for which the values of F_l and F_r have already been given, the values of G_l and G_r are as follows:

	No. of vertebrae				Total no. of backbones
	4	5	6	7	
G_l (left side)	—	43	38	4	85
G_r (right side)	2	47	31	5	85
Sum	2	90	69	9	A. mean = 5·500

and the extent of bilateral asymmetry:

$\frac{G_l}{G_r}$	$\frac{5}{4}$	$\frac{5}{5}$	$\left(\frac{5}{6} \text{ or } \frac{6}{5}\right)$	$\frac{6}{6}$	$\left(\frac{6}{7} \text{ or } \frac{7}{6}\right)$	$\frac{7}{7}$	
No. of backbones	2	40	8	29	3	3	=85

Total of 72

The data show that of the 72 backbones (*ca.* 85 % of the total of 85 examined), in which G_l is the same as G_r, there are 40 (*ca.* 47 % of the 85 examined) in which the count of "tail" vertebrae is 5 along both left and right sides.

The Total Count (T)

The total count (*T*) for the 85 normal backbones in the sample varies thus:

No. of vertebrae				Total no. of backbones	
54	55	56	57		
1	19	57	8	85	A. mean = 55·847

With regard to the 15 "abnormal" backbones, 3 show fusions of vertebrae which make it difficult to determine (*T*) with certainty. In the remaining 12 there is duplication of the neural and haemal spines of one or both of the two vertebrae immediately preceding the terminal urostylar segment, although the centra are normal enough for an uninterrupted count of (T). The results of this count are compared with that for normal backbones:

	No. of vertebrae				Total no. of backbones		
	54	55	56	57			
Backbones with duplicated spines	—	7	5	—	12	A. mean 55·417	Diff. 0·43
Normal backbones	1	19	57	8	85	A. mean 55·847	
Sum	1	26	62	8	97	A. mean 55·794	

It is seen that duplication of spines is associated with a lowering of the total number of vertebrae (T), giving a difference of 0·43 in the value of the mean. If the number of "tail" vertebrae is considered, then it is found that the discrepancy is still more marked:

		No. of "tail" vertebrae				Total no. of backbones	
		4	5	6	7		
Backbones with duplicated spines	G_l	2	9	1	—	12	
	G_r	1	10	1	—	12	
	Sum	3	19	2	—	A. mean 4·958	Diff. 0·542
Normal backbones	G_l	—	43	38	4	85	
	G_r	2	47	31	5	85	
	Sum	2	90	69	9	A. mean 5·500	

By contrast it is to be noted that the mean number of "trunk" vertebrae for the 12 abnormal backbones is 50·459 as against 50·347 for the normal. That is to say, abnormal and normal backbones agree approximately in the number of "trunk" vertebrae, the lowering of the mean value of (T) in association with duplication of spines being almost entirely accounted for by the reduction in the number of vertebrae in the "tail" (cf. Ford, 1933, p. 221).

Variation from Sample to Sample

Having given an idea of the nature and extent of backbone variation among the individuals of an average working sample, it is now proposed to compare the data for a number of different samples. The statistical material to be used in the comparisons was collected and tabulated by Dr A. G. Nichols and colleagues at the Millport Biological Station, to whom I am greatly indebted for their kindly co-operation in a tedious series of observations. The fact that the data were collected by independent workers adds to the interest, since it shows that the successive changes in form along the length of the backbone are sufficiently well defined to be recognized and used by persons other than myself. And so far as I am aware the Millport team experienced no great difficulty in determining with precision the whole series of vertebral counts required. It should be added that the four samples here to be considered were a deliberate selection from a total of seven which they analysed. That is to say, the comparisons to be made are not to be regarded as a critical examination of the actual herring populations occurring in the regions from which the seven samples were drawn, but rather as an illustration of the variation which investigators may expect to observe in carrying out such a regional study.

General particulars of the four samples are given in Table I.

TABLE I

Serial no. of sample	Locality	No. of fish examined	Length (cm.)	Sexual condition
1	Clyde (from Fairlie)	100	21–27 (mainly 22–24)	Mainly stage II
2	Brown Head, Arran	110	23–28 (mainly 23–26)	Stage VI
3	Isle of Man	217	22–28 (mainly 24–26)	Stages VII–II
4	Isle of Man (Port Erin Bay)	105	21–28 (mainly 23–26)	Stage VII

It may be stated in the first place that each of the four samples included an appreciable proportion of abnormal backbones, mainly those in which there was duplication of the neural or haemal processes of the penultimate or antepenultimate vertebrae (Table II).

TABLE II

Serial no. of sample	No. of backbones with fused vertebrae	No. of backbones with duplicated neural and haemal spines	Remainder (normal backbones)
1	1 (1 %)	23 (23 %)	76 (76 %)
2	—	24 (21·8 %)	86 (78·2 %)
3	4 (1·9 %)	66 (30·4 %)	147 (67·7 %)
4	3 (2·9 %)	15 (14·3 %)	87 (82·9 %)

In consequence of the high percentage of backbones with duplicated neural and haemal spines, the total number of vertebrae (T) for each sample is substantially lowered (Table III).

TABLE III

	No. of vertebrae						Total no. of backbones	Total count (T) arithmetic means and differences	
Serial no. of sample	53	54	55	56	57	58			
1. Normal backbones	—	—	5	56	15	—	76	56·132	0·436
Backbones with duplicated spines	—	—	7	16	—	—	23	55·696	
2. Normal backbones	—	1	6	61	18	—	86	56·116	0·408
Backbones with duplicated spines	—	—	7	17	—	—	24	55·708	
3. Normal backbones	—	4	51	81	10	1	147	55·680	0·604
Backbones with duplicated spines	1	7	45	12	1	—	66	55·076	
4. Normal backbones	—	2	38	41	6	—	87	55·586	0·186
Backbones with duplicated spines	—	—	9	6	—	—	15	55·400	

TABLE IV

COUNT A: NO. OF VERTEBRAE WITH AUTOGENOUS HAEMAL ARCH

Serial no. of sample	Count	No. of vertebrae						Total no. of backbones	A. mean
		21	22	23	24	25	26		
1 (Fairlie)	A_l	—	13	44	40	3	—	100	
	A_r	—	11	52	35	2	—	100	
	Sum	—	24	96	75	5	—		23·305
2 (Arran)	A_l	—	10	62	33	5	—	110	
	A_r	—	10	64	32	4	—	110	
	Sum	—	20	126	65	9	—		23·286
3 (I.O.M.)	A_l	3	33	110	64	6	1	217	
	A_r	2	32	120	55	8	—	217	
	Sum	5	65	230	119	14	1		23·173
4 (I.O.M.)	A_l	2	25	49	26	3	—	105	
	A_r	—	23	58	22	2	—	105	
	Sum	2	48	107	48	5	—		23·029

COUNT B: PRE-CAUDAL VERTEBRAE WITH OPEN HAEMAL ARCH

Serial no. of sample	No. of vertebrae						Total no. of backbones	A. mean
	22	23	24	25	26	27		
1 (Fairlie)	1	27	47	17	7	1	100	24·050
2 (Arran)	—	21	58	26	5	—	110	24·136
3 (I.O.M.)	4	81	107	20	5	—	217	23·728
4 (I.O.M.)	4	42	45	13	—	—	104	23·644

COUNT C: NO. OF VERTEBRAE WITH AUTOGENOUS NEURAL ARCH

Serial no. of sample	Count	No. of vertebrae					Total no. of backbones	A. mean
		24	25	26	27	28		
1 (Fairlie)	C_l	—	13	51	33	3	100	
	C_r	—	14	56	26	4	100	
	Sum	—	27	107	59	7		26·230
2 (Arran)	C_l	2	23	57	26	2	110	
	C_r	2	20	62	22	4	110	
	Sum	4	43	119	48	6		26·041
3 (I.O.M.)	C_l	1	23	86	93	14	217	
	C_r	3	19	91	93	11	217	
	Sum	4	42	177	186	25		26·429
4 (I.O.M.)	C_l	—	9	49	39	8	105	
	C_r	2	11	49	39	4	105	
	Sum	2	20	98	78	12		26·371

COUNT D: NO. OF VERTEBRAE WITH DUPLICATED NEURAL SPINE

Serial no. of sample	No. of vertebrae						Total no. of backbones	A. mean
	27	28	29	30	31	32		
1 (Fairlie)	—	—	31	59	9	1	100	29·800
2 (Arran)	—	2	44	48	15	1	110	29·718
3 (I.O.M.)	1	9	78	96	31	2	217	29·705
4 (I.O.M.)	—	7	41	49	7	1	105	29·562

TABLE IV *continued*

COUNT F: NO. OF VERTEBRAE IN "TRUNK"

Serial no. of sample	Count	No. of vertebrae						Total no. of backbones	A. mean
		47	48	49	50	51	52		
1 (Fairlie)	F_l	—	—	15	48	35	2	100	
	F_r	—	1	13	46	39	1	100	
	Sum	—	1	28	94	74	3		50·250
2 (Arran)	F_l	1	—	13	46	48	2	110	
	F_r	—	—	15	55	37	3	110	
	Sum	1	—	28	101	85	5		50·291
3 (I.O.M.)	F_l	—	4	35	129	44	5	217	
	F_r	1	1	44	117	51	3	217	
	Sum	1	5	79	246	95	8		50·048
4 (I.O.M.)	F_l	—	3	20	60	18	4	105	
	F_r	—	2	22	60	19	2	105	
	Sum	—	5	42	120	37	6		49·986

COUNT G: NO. OF VERTEBRAE IN THE "TAIL" GROUP. NORMAL BACKBONES ONLY

Serial no. of sample	Count	No. of vertebrae					Total no. of backbones	A. mean
		4	5	6	7	8		
1 (Fairlie)	G_l	—	20	44	10	2	76	
	G_r	—	25	35	15	1	76	
	Sum	—	45	79	25	3		5·908
2 (Arran)	G_l	—	25	50	11	—	86	
	G_r	—	24	49	13	—	86	
	Sum	—	49	99	24	—		5·855
3 (I.O.M.)	G_l	1	69	75	1	1	147	
	G_r	1	63	77	6	—	147	
	Sum	2	132	152	7	1		5·568
4 (I.O.M.)	G_l	1	34	50	2	—	87	
	G_r	1	31	51	4	—	87	
	Sum	2	65	101	6	—		5·638

THE TOTAL NO. OF VERTEBRAE. NORMAL BACKBONES ONLY

Serial no. of sample	No. of vertebrae					Total no. of backbones	A. mean
	54	55	56	57	58		
1 (Fairlie)	—	5	56	15	—	76	56·132
2 (Arran)	1	6	61	18	—	86	56·116
3 (I.O.M.)	4	51	81	10	1	147	55·680
4 (I.O.M.)	2	38	41	6	—	87	55·586

Table III on p. 163 also shows that the total number of vertebrae for normal backbones is higher in samples 1 and 2 than in samples 3 and 4. Thus, the arithmetic mean values of (*T*) for the former are approximately greater by 0·5 vertebra than those of the latter. A difference of this magnitude would certainly give grounds for suspecting a real populational difference between the fish from Fairlie and Arran and those from the Isle of Man. For whereas only 7 % of the Fairlie and Arran fish had less than 56 vertebrae, the proportion for the Isle of Man fish was over 40 %. For present purposes, however, this question is of no great moment: the main interest in the comparison is to discover the extent to which vertebral counts other than the total (*T*) also vary from sample to sample. Dr Nichol's determinations of the counts *A*, *B*, *C*, *D*, *F* and *G* (as described on p. 156 of this paper) are summarized in Table IV on pp. 164–5, from which the mean values have been extracted (Table V).

TABLE V

		Serial no. of sample							
		1		2		3		4	
Count	Diff.	A. mean	Diff.	A. mean	Diff.	A. mean	Diff.	A. mean	Diff.
A		23·305		23·286		23·173		23·029	
	(*B*-*A*)		0·745		0·850		0·555		0·615
B		24·050		24·136		23·728		23·644	
	(*C*-*B*)		*2·180*		*1·905*		*2·701*		*2·727*
C		*26·230*		*26·041*		*26·429*		*26·371*	
	(*D*-*C*)		3·570		3·677		3·276		3·191
D		29·800		29·718		29·705		29·562	
	(*F*-*D*)		20·450		20·573		20·343		20·424
F		50·250		50·291		50·048		49·986	
G	Normal backbones only	5·908		5·855		5·568		5·638	
T		56·132		56·116		55·680		55·586	

It will be seen that there is a pairing off of the samples, 2 agreeing with 1 and 4 with 3. The mean values for counts *A*, *B*, *D*, *F* and *G*, as well as that for the total count (*T*), are all higher in 1 and 2 than in 3 and 4. The same is true of the differences in the means, viz. (*B*-*A*), (*D*-*C*) and (*F*-*D*). Admittedly, the superiority of the sub-counts in 1 and 2 over those of 3 and 4 is not as great as the difference of 0·5 vertebra between the values of the total count (*T*), but it is nevertheless distinct and consistent. It is interesting, too, that the mean number of "tail" vertebrae (count *G*) plainly confirms the difference between the pairs of samples.

But perhaps the most interesting and significant fact to be learned from the above data is that the mean values of count (*C*) and the difference (*C*-*B*) are not higher but lower—substantially lower—in 1 and 2 than in 3 and 4. Were it not for these exceptions, the data as a whole might well have been explained by the simple hypothesis that the Fairlie and Arran samples (1 and 2) comprised a larger proportion of fish with a total of 56 or 57 vertebrae than the Isle of Man samples (3 and 4). For it would be expected that backbones with a higher total number of vertebrae would, on average, show a higher number of vertebrae in each of its component parts. As matters stand, however, it is necessary to suspect that there is a difference between the fish of the Isle of

Man and those of Fairlie and Arran, *even among those having the same total number of vertebrae*. Accordingly, a comparison has been made to test this, using backbones with a total of 56 vertebrae. The results with regard to the critical count (*C*) are as given in Table VI.

TABLE VI

Serial no. of sample	Count	No. of vertebrae					Total no. of backbones	A. mean
		24	25	26	27	28		
1	C_l	2	16	34	9	—	61	
(Fairlie)	C_r	1	14	32	13	1	61	
	Sum	3	30	66	22	1		$C=25{\cdot}902$
2	C_l	—	10	32	14	—	56	
(Arran)	C_r	—	9	35	11	1	56	
	Sum	—	19	67	25	1		$C=26{\cdot}071$
3	C_l	—	7	29	36	8	80	
(Isle of Man)	C_r	—	5	31	40	4	80	
	Sum	—	12	60	76	12		$C=26{\cdot}550$
4	C_l	—	3	18	16	4	41	
(Isle of Man)	C_r	—	4	19	16	2	41	
	Sum	—	7	37	32	6		$C=26{\cdot}451$

It is seen that the mean value of (*C*) for the Isle of Man samples is definitely higher than that for the Fairlie and Arran samples. There is no need here to give the results of similar comparisons between backbones having a total of 55 or 57 vertebrae, although of course this would be necessary in an actual regional survey.

MERISTIC AGREEMENT BETWEEN THE BACKBONE AND OTHER BODILY ORGANS

All the details and data discussed in the foregoing sections are in respect of individual variation in the form of the backbone itself, apart altogether from variation in other bodily characters. But in the herring, as in any other animal with a segmented body, the relation between successive segments of the backbone and the corresponding segments of other organs is a problem of great interest. For if the bones, muscles, and scales (among other structures) along the full length of the body, or some part of it, are in meristic conformity, then a count of the one is, in effect, a count of the other. And not this alone, for the count itself is a statistic of the basic plan upon which the whole body is organized. Such a statistic might prove of especial service and convenience in the kind of population studies with which this paper is concerned.

Relevant data are given in Fig. 3, which shows the extent of agreement between the backbone, myocommata, fin-radials and keeled scales in a herring which has been dissected and stained in alizarin. It is seen that the backbone consists of 55 vertebrae between the skull and the terminal urostylar segment. Meristic agreement is to be observed in the anterior part of the body. Dorsal to the backbone, the neural spines of the 1st to the 17th vertebrae regularly alternate with a series of bony rods, described by Hillier[*] (1932, p. 98) as the root-bones of a fin now submerged. Immediately posterior to the last of these root-bones, where the first of the radials of the dorsal fin makes its appearance, the conformity with the neural spines ceases, so that the whole base of the dorsal fin lies within the span of the neural spines of the 17th to 30th vertebrae. Ventrally to the backbone, the ribs (the first of which is attached to the 3rd vertebra), myocommata, and keeled scales are in step, backward to the anus. Behind the anus, the haemal processes of the vertebrae from the 38th to the 46th or 47th, are out of step with the radials of the anal fin.

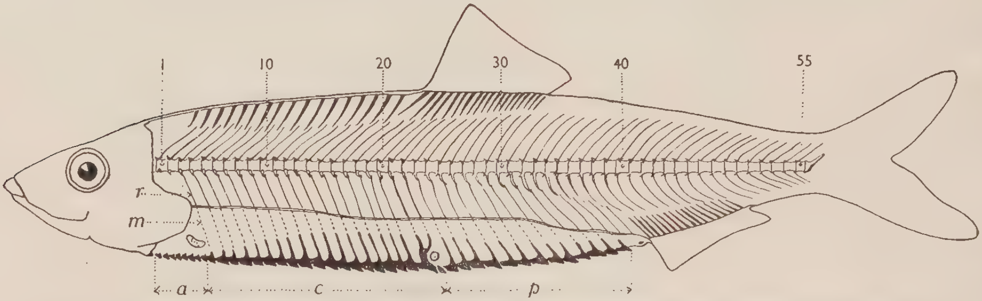

Fig. 3. Left side of a herring to show the extent of meristic agreement between the backbone, fin-radials, myocommata and keeled scales (see text). *r*, rib. *m*, myocomma. *a*, *c* and *p*, anterior, central and posterior groups of keeled scales.

Counts of the Keeled Scales

Along the ventral edge of the body of the herring from the throat backwards to the anus there is a median row of scales. These "keeled scales" have been used in the past as the basis of statistical counts in biometric studies. Usually a distinction has been drawn between the K_1 scales which lie in front of the pelvic fins, and the K_2 scales behind the pelvics. The K_2 count is the one most generally adopted for statistical work, and the first scale to be included in the count is situated between the insertions of the pelvics (see Johansen, 1919, Fig. 9).

Now without in the least degree criticizing the use of K_1 and K_2 counts as statistics of variation within the section of the body in which they are situated, it is of no little interest to consider the number and disposition of the keeled scales in relation to the myocommata, ribs and vertebrae with which they are observed to be in association. In Fig. 3 the keeled scales are marked off into three groups—an anterior group (*a*) of 8, a central group (*c*) of 20, and a posterior one (*p*) of 13, making a total of 41 scales in all between the throat and the anus. For purposes of description it is convenient to deal first with the scales of the central group, which begins at the 9th scale. This 9th scale is seen to be in register with a myocomma, and with the first of the ribs, which is attached to the 3rd vertebra. Proceeding backwards along the series, the 10th, 11th, ..., to the 26th scale are similarly associated with their respective myocommata and the successive ribs of the 4th, 5th, ... to the 20th vertebra. The 27th scale is a compound one having lateral processes which loop round the base of the pelvic fin and, at their distal end break into two and make register with two myocommata and the underlying ribs of the 21st and 22nd vertebrae. There remains the 28th scale from the throat, which appears to be attached anteriorly to the posterior end of the preceding scale. Summarizing, the 20 keeled scales from the 9th to the 28th, both inclusive, form a central group which is in meristic agreement with 20 myocommata and the ribs of 20 vertebrae from the 3rd to the 22nd. It may also be added here that the vertebrae referred to have autogenous parapophyses, the 22nd vertebra being the hindermost one to show this character.

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[*] Hillier's treatise, entitled *A Theory of the Formation of Animals*, contains a very useful and detailed description of the anatomy of the herring, based on personal observations. This footnote will serve to draw attention to a work which may have escaped the notice of many fishery investigators.

The posterior group of 13 scales commences with the 29th scale from the throat and ends with the 41st just anterior to the anus. Each of these is in register with a myocomma and a vertebra, the first being in register with the 23rd vertebra (the first to have its haemal arches continuous with the centrum and the ribs attached by ligament—the "false" ribs of Hillier, 1932, p. 94) and the last in register with the 35th vertebra.

With the central and posterior groups thus orientated, it is only necessary to state that the anterior group consists of 8 scales of comparatively small size which are without conspicuous lateral processes and are situated in front of the first rib.

The foregoing data relate to a selected specimen from a sample of young herrings of the "O" age-group caught at Plymouth. After removing the scales from the sides of the body in the neighbourhood of the pectoral fins, pelvic fins, and keeled scales, each fish was split longitudinally to expose the backbone and then stained in alizarin. Thus prepared, the keeled scales, myocommata, ribs and vertebrae could be easily counted *in situ* under a dissecting microscope. Only 25 fish in all were examined, but these were sufficient to show that appreciable variation from fish to fish had to be taken into account. The total number of keeled scales varied from 41 to 45, while for the three groups into which the total may be divided the range of variation was from 7 to 11 in the anterior group, 19 to 21 in the central group, and 13 to 16 in the posterior group, with frequencies as shown in Table VII.

But it was also found that in 15 of the total of 25 fish the number of keeled scales in one or other of the central and posterior groups was greater by 1 than the number of myocommata (and ribs) with which the scales were in register. In each instance the breakdown of the normal association of 1 keeled scale with 1 myocomma (and rib) occurred in the region adjacent to the insertion of the pelvics. In 2 of the 15 specimens the last two scales of the central group were in association with a single myocomma, while in the remaining 13 the first two scales of the posterior group were covered by one myocomma only (see Fig. 4). The significance of these observations in connexion with the K_2 count will not have escaped notice, for it implies that the K_2 count is not a constant function of the number of myocommata and vertebrae with which the keeled scales counted are in association. Whether or not this fact affects the results of any particular biometric investigation is immaterial in the present consideration, but it is nevertheless one which should be borne in mind.

TABLE VII

		No. of keeled scales					
		41	42	43	44	45	A. mean
No. of specimens:	Total count	6	4	5	6	4	42·92
	Anterior group	7	8	9	10	11	
		1	8	8	7	1	8·96
	Central group		19	20	21		
			1	17	7		20·24
	Posterior group		13	14	15	16	
			10	13	1	1	13·72

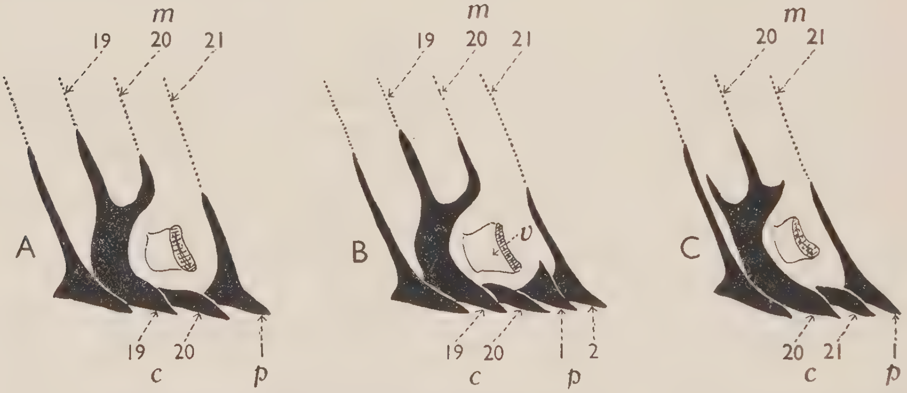

Fig. 4. Keeled scales in the neighbourhood of the pelvic fins. A, the last two scales of the central group (*c*, 19 and 20) and the first scale of the posterior group (*p*, 1) are in register with the 19th, 20th and 21st myocommata; B, a common variation in which the last two scales of the central group (*c*, 19 and 20) and the first two of the posterior group (*p*, 1 and 2) are associated with the 19th, 20th and 21st myocommata; C, a less common variation in which the last two scales of the central group (*c*, 20 and 21) are in register with a single myocomma (20th) instead of two as normally.

DISCUSSION

As a concluding section of this paper, the results given in preceding pages will be considered in their bearing upon the Resolutions adopted by the Lowestoft meeting. An agreed scheme for the detailed study of backbone variation in as many biological groups as possible is clearly desirable. I cannot recommend that the work should be restricted to a selected few of the vertebral counts described above, because I firmly believe that our past persistence in doing so has prevented us from making progress at the rate commensurate with the amount of solid and tedious labour expended. Long before this we should have undertaken the detailed investigation of the herring backbone in all its parts and of variation from group to group which is only now being contemplated, and without which we cannot make much headway in the future. It is not suggested that past work has been entirely unprofitable, for much useful information has been acquired concerning the variation of the total count (T) in particular, but much of it has proceeded in the absence of an adequate knowledge of the nature and extent of vertebral variation, with a consequent waste of effort and the accumulation of a mass of bewildering data. This, at any rate, is a personal conviction, based on personal experience.

In planning the scheme, considerations of economy in labour and material must be treated as secondary to the requirements for detecting differences between the biological groups. All the alternative counts along the backbone, as well as combinations of counts and differences between counts, should be examined as potential indicators of the differences between groups. The working samples should be fully representative of the biological groups from which they are drawn and large enough to enable comparisons to be made between backbones having the same total number of vertebrae. Recognized statistical methods should be followed in making the comparisons.

Provided these main points are observed, the details of the scheme can with advantage be left to the discretion of the workers who are to carry it through. If all the biological groups are to be studied, as eventually they must, numbers of workers in the different countries interested in the herring fisheries will need to co-operate, but I do not think the amount of work which each must perform should prove excessive. One worker with the aid of a laboratory attendant can prepare 250 backbones by boiling and cleaning in a single day, and the determination of the vertebral counts can be carried out at his convenience on the dried preparations, which will keep in good condition without preservation for many months if necessary. It will be found most helpful for the subsequent analysis of the data to enter all the counts for each fish on a separate card bearing the particulars of the sample and the serial number of the fish. Frequency tables according to any desired criteria can then be quickly compiled by the simple process of dealing out the cards into the correct piles and counting the number in each pile. This can be done without clerical assistance and enables the investigator to try out many alternative comparisons with the minimum of trouble and sub-tabulation.

I think the question of the best use to be made of the count of keeled scales is one of some importance, which should be included in any proposed scheme. The data given in this paper are, of course, inadequate for final decision; but they raise points of great interest, particularly with regard to the basic metamery of the herring and the relation between keeled scale and vertebral counts.

Finally, I strongly recommend that a determined effort should be made to discover the morphological characters which have been referred to in this paper as the "hallmarks" of the biological groups—those characters which will reveal sub-specific identity by inspection of individual fish, irrespective of circumstances of time and place of capture. Given a representative sample from each of the biological groups, I am of the opinion that careful comparisons, vertebra by vertebra, would bring to light differences of the greatest interest and practical usefulness.

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THE GENITAL DUCTS OF SOME BRITISH STENOGLOSSAN PROSOBRANCHS

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(Text-figs. 1–6)

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INTRODUCTION

It has long been known that in the stenoglossan Prosobranchia the ventral pedal gland plays some part in the formation of the egg capsule, but the exact functions of the various parts of the genital ducts have not hitherto been investigated. The ventral pedal gland was originally taken to be the external opening of a water vascular system, and Carrière (1882) was the first to show that this structure was really a gland. Cunningham (1899) ascribed to it the function of producing the chitinous capsules around the eggs which had been passed into it from the oviduct in *Buccinum* and *Murex* (= *Ocenebra*), and of fixing them to the substratum. Simroth (1896–1907) after first agreeing with this opinion ultimately concluded that the capsules were formed in the oviduct and then passed to the pedal gland. His theory was originally rejected by

Pelseneer (1910), who found capsules in the pedal gland of *Purpura* (=*Nucella*) *lapillus* which, owing to their thin, transparent, colourless walls, so unlike the final product, suggested that secretion from the gland was responsible for forming them; this view was quoted by Thiele (1925). Later, however, Pelseneer (1926) obtained capsules from the terminal region of the oviduct of *Nassa* (=*Nassarius*) *reticulata*, which proved that the origin of the capsule must be sought in the genital ducts, though the pedal gland may complete its moulding and harden it. Three years later Ankel (1929) in his observations on *N. mutabilis* and *N. reticulata* compared egg capsules taken from the oviduct with those completed by the pedal gland, and proved conclusively that the gland merely moulds and hardens the secretion which is laid down around the egg mass in the genital ducts. Ankel (1929, 1935, 1937) further suggested that the two sutures easily visible on the surface of the egg case of *N. mutabilis*, *N. reticulata*, *Nucella lapillus* and also of *Lamellaria perspicua* were due to the fact that the ducts in which the capsules are secreted are bilobed. Ankel's conclusions agree in every detail with the observations here described, but his papers include no study of the local manufacturing of the various parts of the egg case nor of the manœuvring of eggs and sperm within the ducts. A complete account of the structure and function of the genital ducts of both sexes is given in the following pages.

The work was carried out at Birkbeck College, Royal Holloway College, and the Plymouth Marine Laboratory. I wish to express my thanks to the University of London and to the British Association for the use of their tables at Plymouth.

The animals of which the genital ducts were investigated included *Ocenebra erinacea* (L.), *Nucella lapillus* (L.), *Buccinum undatum* L., *Nassarius reticulatus* (L.) and *N. incrassatus* (Ström), using the names given by Winckworth (1932). The animals were examined alive by means of a binocular microscope. The tissues were difficult to manipulate owing to their extremely glandular nature, and it was found best to fix them in Susa and Keilin's (1921) modification of Bouin. The distribution of different types of glands in the duct was most easily demonstrated by staining the whole structure in an alcoholic solution of thionin.

THE DUCTS OF THE MALE

Ocenebra erinacea

From the testis, situated on the columellar side of the visceral mass, the coiled vas deferens passes anteriorly in a superficial position. It leads beneath the gut and the pericardium to the right posterior corner of the mantle cavity and here opens into the prostate gland. The latter runs parallel with the rectum to the opening of the mantle cavity and from it a narrow duct passes along the right side of the head to the penis, which lies behind the right tentacle. On account of histological and physiological differences the duct leading to the prostate is divisible into two regions—a long coiled posterior part, which in the breeding season is distended with sperm, thus functioning as a vesicula seminalis, and short straight ciliated part leading beneath the intestine and the pericardium to the prostate, and separated from the vesicula seminalis by a sphincter. The two regions correspond to the vesicula seminalis and the vas deferens described by Linke (1933) in *Littorina littorea*, *L. obtusata* and *L. rudis*, and there is close agreement also in histological detail. In the vesicula seminalis the epithelium consists of columnar ciliated cells resting upon a basement membrane, beneath which a few circular muscles are developed. The basal nuclei of the columnar cells are spherical or irregular in outline, each with a nucleolus, and above them the cytoplasm is vacuolated. During the breeding season at least, many of the vacuoles contain lightly staining spherules, whereas in others ingested sperm may be found, for, as in the species of *Littorina* previously mentioned, the vesicula seminalis serves as an area for sperm absorption. The absorbed sperm are digested and the spherules may be regarded as their breakdown products. Relaxation of the sphincter at the entrance to the lower part of the duct, the true vas deferens, allows the contents of the vesicula seminalis, in which the sperm are under pressure, to pass to the prostate. The movement of sperm through the vas deferens is assisted by the thick layer of cilia borne by the columnar epithelial lining. The cilia arise from basal granules, and from these intracellular fibrillae run deeply into the cytoplasm. The epithelium is surrounded externally by circular muscles which are more numerous than in the vesicula seminalis. Close to its opening into the prostate the vas deferens gives off a short diverticulum towards the pericardium, and the blind end of the diverticulum is connected with a slight prominence on the pericardial wall by a band of dense connective tissue and muscle fibres. This strand reaches the pericardial wall close to the renopericardial aperture and lies in a comparable position to the gonopericardial duct of the female. In *Littorina*, Linke (1933) describes a similar connective tissue strand passing from the vas deferens to the pericardium though no diverticulum is mentioned. In *Calyptraea sinensis* (Giese, 1915), animals changing from male to female develop a gonopericardial duct, and this first appears as a strand of dense mesenchyme bridging the gap between the vas deferens and the pericardium. It therefore appears that in the male of *Ocenebra erinacea* this is the remnants of a gonopericardial duct.

The lateral walls of the prostate (Fig. 1) are thickened by a profuse development of glands (CGC) beneath the ciliated epithelium (CE), and in transverse section the lumen (L) appears as an extensive longitudinal slit between these lobes. The vas deferens leads to the narrow ventral surface of the gland and its entrance is guarded by a sphincter. If the mantle cavity of *Ocenebra* be opened to display the prostate it will be seen that the gland communicates with the posterior extremity of the mantle cavity by a slit-like aperture along the thin ventral wall. The aperture lies between two projecting flaps of tissue which in front of it fuse and are continued along the length of the ventral surface of the gland as a slight ridge. Sections show that the projecting lips of the orifice are ventral extensions of the epithelial lining of the prostate beneath which no glands are present, and anteriorly they are joined one to the other to close the prostate (FE) and to form the projecting ridge. In *Littorina* the prostate is composed of two similar lateral glandular lobes, but it is open throughout its length along the ventral surface. Thus *Ocenebra* demonstrates the formation of a closed prostate from the open type of *Littorina*, and the opening into the mantle cavity is due to the incomplete fusion of the two lobes. The significance of this opening will be dealt with later. In the vicinity of the opening the cilia beat into the prostate, at right angles to the aperture. Elsewhere in the gland they cause an anteriorly directed current.

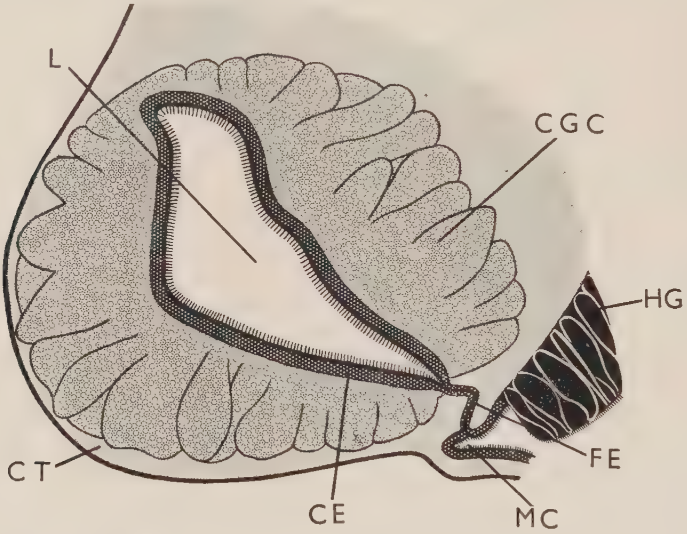

Fig. 1. *Ocenebra erinacea.* Transverse section through the prostate: camera lucida drawing (× 56).

CE, ciliated epithelium; CGC, cluster of subepithelial gland cells; CT, connective tissue; FE, epithelia which fuse to close the prostate; HG, hypobranchial gland; L, lumen of prostate; MC, mantle cavity.

The subepithelial gland cells of the prostate are grouped in spherical clusters (CGC), the ducts from a single cluster running parallel with one another to open between the ciliated cells. Each gland cell has a round basal nucleus with a prominent nucleolus. The cytoplasm is packed with granules which stain lightly with iron haematoxylin, except those around the nucleus which stain more densely. After azan the contents are yellowish in the vicinity of the nucleus and in the upper parts of the cell varying shades of purple. The azan stain shows that between these gland cells are scattered mucous cells. The columnar ciliated cells are compressed laterally by the ducts of the glands; their elongated nuclei lie towards the centre of the cytoplasm. The cilia arise from basal granules, and intracellular fibrillae pass from these into the deeper regions of the cell, which also contains numerous granules. In the region of fusion between the two ventral lips of the prostate the cells of one side are in close contact with those of the other and no trace of cilia can be seen between, so that, in transverse section, a double row of cells appears to lead from the lumen to the mantle cavity (FE). These cells are cubical, with spherical nucleolated nuclei in the centre of the cytoplasm. At the opening into the mantle cavity the two sheets of epithelium separate; their cells become tall and columnar, like the cells lining the prostate which they resemble in all other features. Among them are a few mucous cells.

From the ventral surface of the prostate arises the ciliated tube which runs up the right side of the head to the penis. In *Littorina* this duct is represented by a deep groove running along a raised muscular ridge, and the edges of the groove can be brought close together to form a closed channel. In *Ocenebra* the edges have fused to form a tube, and evidence of this fusion is seen in sections by the two joined strips of epithelium, which are continuous with those of the prostate and lead from the lumen to the exterior. They are thrown into longitudinal folds and possess the same histological structure as in the previous region. In like manner the channel leading up the penis to its tip has been closed in *Ocenebra*, whereas it is open in *Littorina*.

The duct to the penis runs up the side of the head along a slight ridge which a layer of transverse muscles separates from the underlying tissues. The epithelium is thrown into longitudinal folds owing to the differences in the heights of the columnar ciliated cells of which it is composed. The nuclei are round, oval or irregular and lie towards the middle of the cells which, distally, contain granules; intracellular fibrillae pass from the basal granules of the cilia to the region of the nuclei. Beneath the basement membrane is a well-developed layer of circular muscles.

The penis is slightly flattened dorsoventrally and its duct is not centrally placed, but lies towards the outer edge. The histological structure of the epithelium is similar to that of the preceding duct; no gland cells are present. At its tip the two layers of fused epithelia separate to form the penial aperture. A layer of circular muscles lies beneath the epithelium of the duct and extends beneath the fused strips of epithelium. Throughout the thickness of the penis muscle fibres penetrate the connective tissue—longitudinal muscles run from base to tip, a layer of circular muscles runs under the outer epithelium, and beneath these dorsoventral and oblique muscles are developed. Blood spaces are numerous and the penial nerve occupies a central position.

Nucella lapillus

The gross morphology of the male reproductive system of *Nucella lapillus* is similar to that of *Ocenebra erinacea*, and between the two there is a close correspondence in histological detail. Only the few points in which they differ will be mentioned. In the short vas deferens, which leads from the vesicula seminalis to the prostate, no trace of a gonopericardial duct can be found, and the muscles underlying the vas deferens are more strongly developed than in *Ocenebra*. The prostate is smaller and the spherules filling its gland cells, of which there is only one type, stain lightly with mucicarmine and blue with azan. The opening of the prostate into the mantle cavity is reduced to a minute aperture, and along the whole length of the genital duct anterior to this point can be seen the two strips of epithelium which fuse to close it. That part of the duct which runs from the prostate up the right side of the head to the penis has, underlying it, a thicker layer of circular muscles, and the penis is more dorsoventrally flattened than in *Ocenebra*.

Nassarius reticulatus

The structure of the male genital duct of *Nassarius reticulatus* differs in some essential features from that of *Ocenebra* or *Nucella*. The upper part of the vas deferens is greatly coiled and functions as a seminal vesicle; in the breeding season it can be traced below the surface of the visceral mass distended with sperm. The epithelium resembles that of *Ocenebra*: in like manner it is capable of ingesting sperm from the lumen. The true vas deferens is a straight duct which leads to the upper extremity of the mantle cavity on the right side, and rarely contains sperm except during the time of copulation. It is separated from the preceding region by a sphincter, and throughout its course a thick coat of circular muscles underlies the columnar ciliated epithelium which lines it. A slight thickening in the connective tissue between the vas deferens and the pericardium suggests the remains of a gonopericardial duct.

From the vas deferens the genital duct leads forward along the right side of the mantle cavity; its course is approximately parallel to that of the rectum. At the opening of the cavity it passes up the right side of the head to the penis. In *Ocenebra* the posterior region, traversing the length of the mantle cavity, is represented by the prostate. It has a much enlarged lumen and thick glandular walls, and the anterior region is a narrow ciliated duct. In *Nassarius* no such differentiation occurs, and from the vas deferens to the penis the duct is narrow, of about the same diameter as the former, and is of uniform histological structure—it is lined by an epithelium of gland cells alternating with ciliated cells and no subepithelial glands occur. The opening of the vas deferens into this anterior glandular region, the whole of which may be termed the prostate, is marked by a projecting circular fold of ciliated epithelium, and a sphincter is capable of closing the aperture completely. From this point, at the posterior extremity of the prostate, a short ciliated duct leads to the mantle cavity and, on dissection, its opening can be seen between the anterior margin of the kidney and the genital duct. It corresponds to the slit-like opening of *Ocenebra*. The duct has thick muscular walls and its external opening may be closed by a sphincter. Anterior to it the genital tract has no connexion with the mantle cavity, and there is no indication of its formation from an open groove as in *Ocenebra*.

Throughout its length the prostate is surrounded by a thick layer of circular muscles, and during copulation the spermatozoa are conveyed with great rapidity along it by peristalsis aided by the cilia. The gland cells which alternate with the ciliated cells of the epithelium have highly vacuolated protoplasm. The vacuoles contain spherules which tend to dissolve on fixation, but which stain lightly with iron haematoxylin and pale blue with azan. The spherical nuclei, each with a prominent nucleolus, lie towards the base of the cytoplasm. The cilia arise from basal granules and from these intracellular fibrillae can be traced to the central region of the cell. Minute orange or yellow pigment granules are scattered in the distal half of each cell and the oval nuclei lie towards the base. That part of the duct which runs up the side of the head to the penis is situated on a slight ridge, and this is separated from the underlying tissues by a sheet of transverse muscles.

The penis is flattened dorsoventrally and its duct is central. The histological character of the lower part of the duct is exactly similar to that of the prostate, but towards the mid region the gland cells disappear, and distally the duct is lined by a columnar ciliated epithelium with cells of uniform height. As in the preceding region of the genital tract a thick layer of circular muscles underlies the epithelium, and spermatozoa are conveyed to the terminal opening by peristalsis and ciliary action. In the subepithelial connective tissue of the terminal part of the duct there are numerous mucous cells, their necks passing between the ciliated cells. The musculature of the penis differs from that of *Ocenebra*: beneath the external epithelium is an outer layer of circular and oblique muscles and an inner layer of longitudinal fibres, and well-developed blood spaces run laterally through the length of the penis. The central region, containing the duct with its thick muscular coat, the penial nerve and other blood spaces, is separated from the outer region by a layer of circular muscles, and dorsoventral muscles penetrate every part of the connective tissue.

Buccinum undatum

The male genital duct of *Buccinum undatum* (Fig. 2) resembles most closely that of *Nassarius reticulatus*. The coiled posterior part of the vas deferens acts as a vesicula seminalis (VS). It is covered by a ciliated epithelium and the cytoplasm of the cells is vacuolated. The distal vacuoles may contain ingested sperm, and in others may be seen brownish granular concretions which, as in *Ocenebra*, are probably the products of sperm digestion. A thick muscular coat is developed below the basement membrane and this consists of an inner circular layer and outer longitudinal fibres. The duct straightens just before it enters the posterior end of the mantle cavity and the musculature increases in thickness. This short region which passes to the mantle cavity is the true vas deferens (VD), into which the sperm are liberated during the breeding season and through which they are passed mainly by muscular activity. Ventral to the kidney aperture the vas deferens opens into a thicker region of the genital duct (PR), which passes forward along the right side of the body, beneath the rectum (R), and projects from the surface of the floor of the mantle cavity as a prominent ridge. At the mouth of the cavity it passes dorsally to the base of the penis (P) which is situated on the right side of the neck. The region of the duct which passes through the mantle cavity to the penis is of about the same diameter throughout, although there appears fairly constantly a constriction (CO) just about the point where the duct passes on to the side wall of the proboscis sheath.

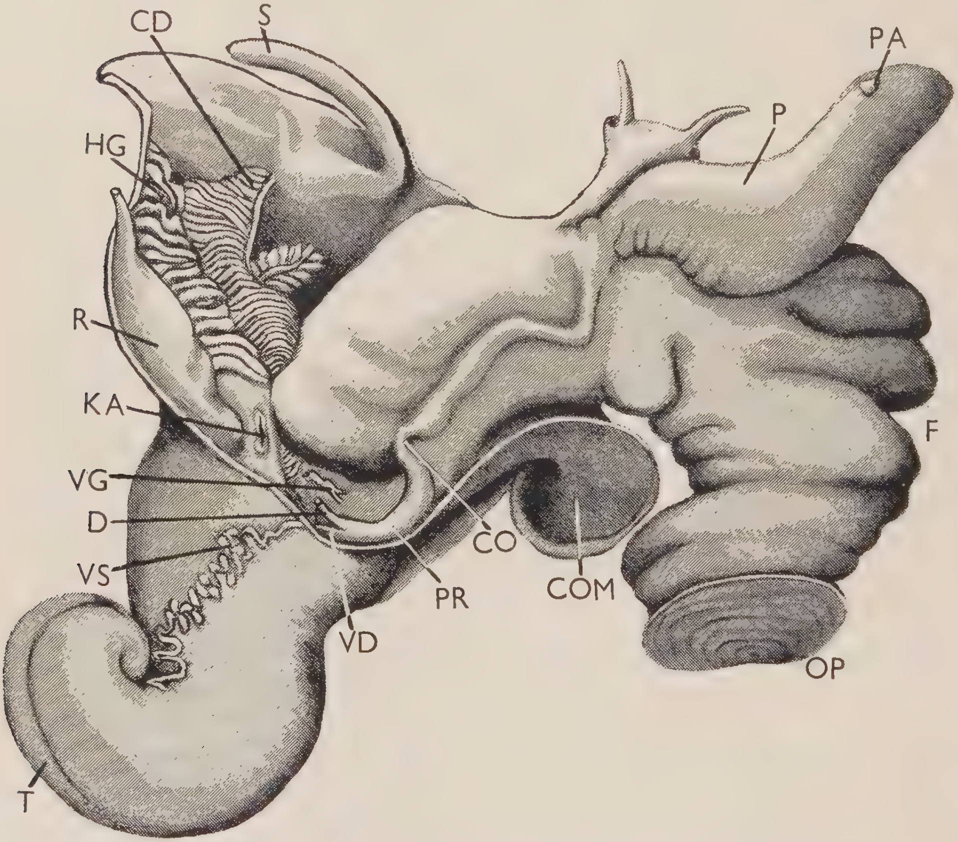

Fig. 2. *Buccinum undatum.* Dissection to display the male genital duct, the mantle cavity having been opened along the right side (× about $1\frac{3}{5}$).

COM, columellar muscle; CO, constriction; CD, ctenidium; D, duct leading from the prostate to the mantle cavity; F, foot; HG, hypobranchial gland; KA, aperture of kidney; OP, operculum; P, penis; PA, papilla; PR, prostate; R, rectum; S, siphon; T, testis; VD, vas deferens; VG, visceral ganglion; VS, vesicula seminalis.

If the mantle cavity of a male *Buccinum* be opened to expose the prostate (Fig. 2) it will be seen that from the posterior end of this gland and on the left side there arises a narrow duct (D). This passes backwards and to the left to open to the mantle cavity. The opening is alongside the visceral ganglion.

The presence of this structure was not mentioned by Dakin (1912) in his description of the male genital system. It is lined by a columnar ciliated epithelium in which mucous glands are scattered. Beneath the epithelium is a very thick layer of circular and longitudinal muscles. The opening of the vas deferens into the prostate is situated on a small papilla immediately anterior to the opening of the duct. Internally the walls of the prostate are folded longitudinally and covered by a columnar ciliated epithelium. A layer of circular muscles, 200μ thick, underlies the basement membrane, and between the muscles pass the ducts of a still deeper layer of gland cells. The glands are of two types. In one the cytoplasm is filled with small spherules which stain lightly with iron haematoxylin and red with azan. The nuclei are spherical and each has a prominent nucleolus. The second type of cell is a mucous cell. The ducts are long, exceeding 300μ, and they pass between the epithelial cells to open into the lumen of the gland. During the breeding season, when the gland is well developed, the distal tips of the ducts are swollen with secretion and compress the ciliated cells. Circular and oblique muscle fibres pass between the glands and surround the prostate externally.

The duct which passes through the large flattened penis (P) opens at the apex of a papilla (PA) which is subterminal and on its upper edge. The duct is lined by a columnar ciliated epithelium and surrounded by a thick layer of circular muscle. About the middle of the penis the duct measures only 180μ in diameter, but towards the papilla it is further reduced to a very fine tube and the thickness of the muscular coat is correspondingly decreased.

The Ducts of the Female

Nucella lapillus

Owing to the ease of obtaining material the female genital system and the formation of the egg capsules has been studied in greater detail in *Nucella lapillus* (Figs. 3, 4, 5*a* and 6) than in *Ocenebra erinacea.*

The ovary (Fig. 3, O) spreads over the surface of the digestive gland in the visceral mass, and from it a thin-walled oviduct (Figs. 3, 4, OD) leads forwards and ventrally on the right side of the viscera. The anterior region can be seen through the integument and has the appearance of a large blood vessel. At a point below the kidney it opens into the albumen gland (Fig. 3, AG) which turns abruptly dorsalwards, making an acute angle with the oviduct, and then recoils on itself passing ventrally to open into the capsule gland (Figs. 3, 4, OAC). At the junction of the oviduct with the albumen gland arises the gono-pericardial duct. The capsule gland leads forward on the right side of the mantle cavity and, on dissection, it can be seen as an opaque white or yellowish mass which is divided into right and left glandular lobes (Figs. 3, 4, RLC, LLC). These lobes are joined dorsally and ventrally by a comparatively thin and narrow wall forming a dorsal and ventral suture, so that in transverse section the lumen of the gland has the appearance of a dorsoventral slit (Fig. 5*a*, L).

From the narrow ventral wall, where gland cells are absent, arise two longitudinal folds of tissue (Figs. 4, 5*a*, RL, LL). These form between them a longitudinal channel (Fig. 5*a*, VC). The left longitudinal fold (LL) is better developed than the right (RL), over which it bends and which it may completely envelop. Thus the ventral channel may be closed off from the lumen of the capsule gland and form a functionally closed duct. Posteriorly the channel leads through a short duct (Fig. 4, DIG) to a deep brown glandular mass situated between the capsule and albumen glands. This is the ingesting gland (Fig. 3, IG) and its narrow duct is surrounded by a thick muscular coat. The albumen gland opens into the posterior ventral wall of the capsule gland on the right side of the ventral channel (Fig. 4, OAC). Anteriorly the capsule gland opens into a muscular vestibule (V), and through this the ventral channel leads into the bursa copulatrix (BC), which when filled with sperm bulges into the vestibule. The short vagina (VA), into which the penis is inserted to deposit sperm in the bursa, and from which the fully formed egg capsules pass from the vestibule to the exterior, opens on the right anterior extremity of the mantle cavity, ventral to the anus; the opening is surrounded by a sphincter.

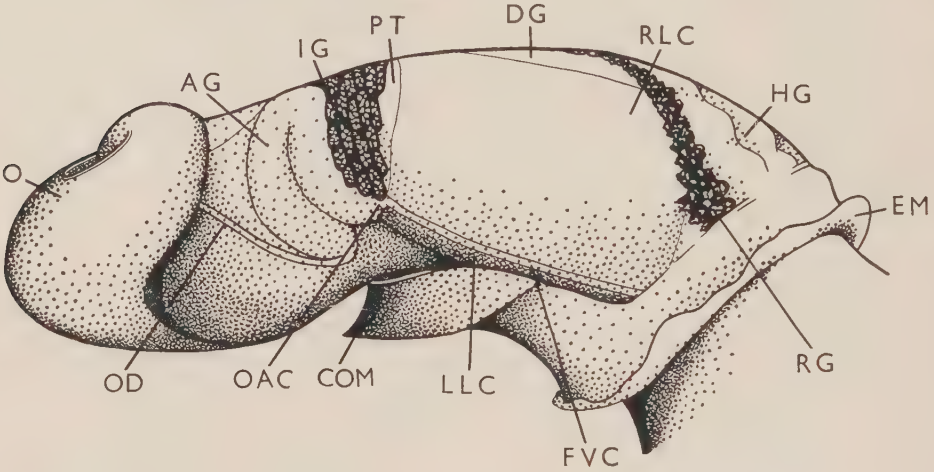

Fig. 3. *Nucella lapillus*. Visceral hump seen from the right side after the removal of the shell (× 6).

AG, albumen gland; COM, columellar muscle; DG, gland cells of the dorsal wall of the capsule gland; EM, free edge of mantle; FVC, floor of ventral channel; HG, hypobranchial gland; IG, ingesting gland; LLC, left lobe of capsule gland; O, ovary; OAC, opening of albumen gland into capsule gland; OD, oviduct; PT, posterior tip of capsule gland; RG, rectal gland; RLC, right lobe of capsule gland.

As the oviduct approaches the albumen gland its glandular lining is replaced by a columnar ciliated epithelium, thrown into longitudinal folds by variations in the thickness of the underlying connective tissue. A similar epithelium is found in the gonopericardial duct. This opens into the pericardium by an inconspicuous ciliated funnel, and beneath the basement

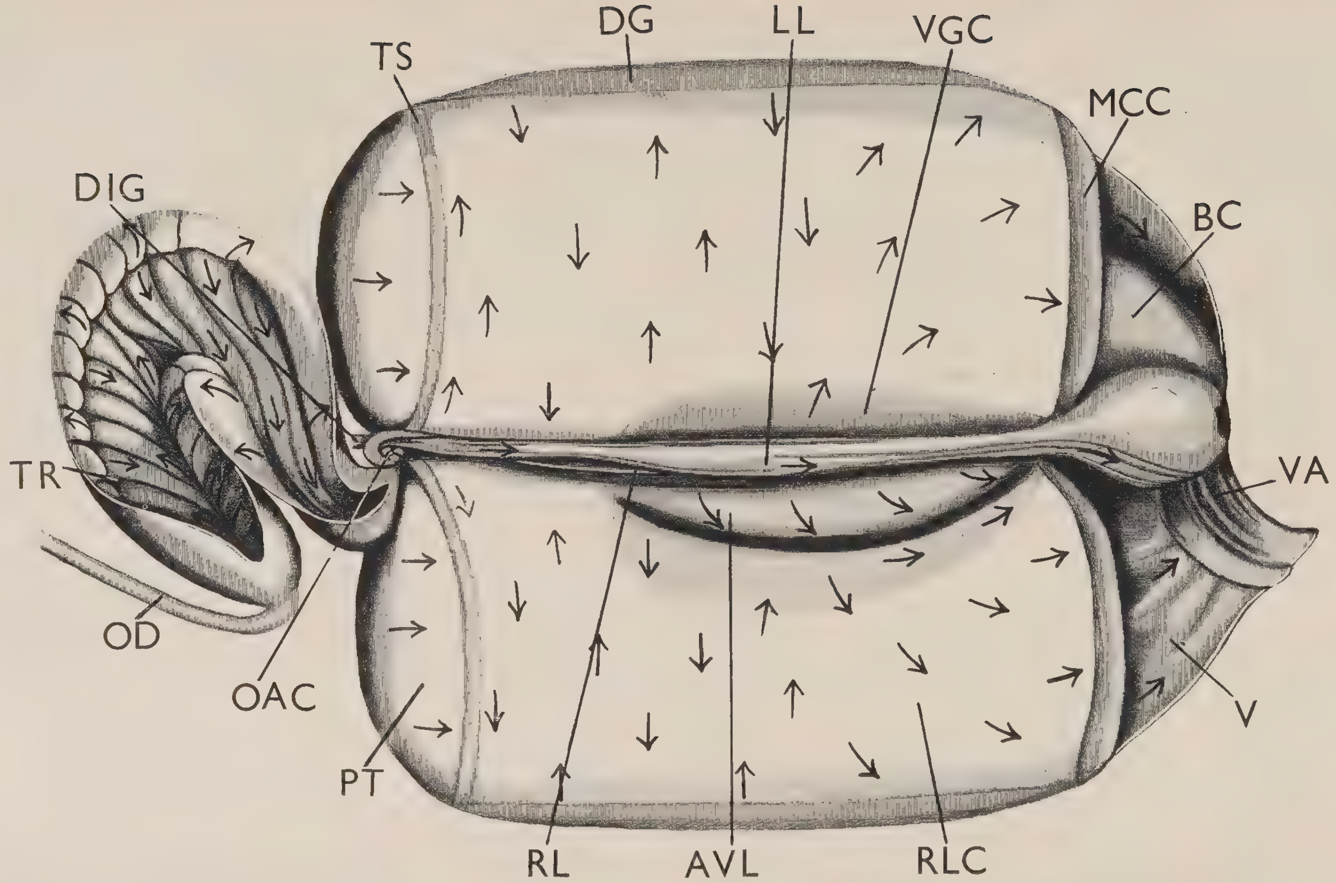

Fig. 4. *Nucella lapillus.* The female genital duct. The muscular vestibule and the capsule gland have been opened by a dorsal longitudinal incision and the albumen gland by a longitudinal incision along the right side. Arrows show ciliary currents. (× about 12)

AVL, anteroventral lobe of capsule gland; BC, bursa copulatrix; DIG, duct of ingesting gland; LL, left longitudinal fold; MCC, mucous cells of the anterior border of the capsule gland; RL, right longitudinal fold; TR, transverse ridges on the wall of the albumen gland; TS, transverse muscular strips; V, vestibule; VA vagina; VGC, anteroventral gland cells. Other letters as in Fig. 3.

membrane of the epithelium are circular and scattered longitudinal muscle fibres; the musculature is well developed around the pericardial opening which it may close completely. A sphincter also surrounds the opening of the oviduct into the albumen gland.

When the shell is removed from a living *Nucella* the opaque, white albumen gland (Fig. 3, AG) is seen through the integument. It is laterally compressed and its walls are ridged transversely (Fig. 4, TR), owing to variations in the thickness of the subepithelial glandular tissue. The gland is ciliated throughout: ciliated cells line the lumen and these are constricted by the ducts of the underlying gland cells which open between them, and by epithelial gland cells which in some parts alternate with them. The nuclei of the ciliated cells are elongated and lie towards the base of the cytoplasm, or they may be pushed towards the distal end of the wedge-shaped cells. Living material shows that the gland cells contain small colourless spherules or granules. The subepithelial cells are grouped in clusters and the ducts from each cluster run parallel with one another to open between the ciliated cells. The nuclei lie towards the base of the cytoplasm and are oval or circular in outline, each with a nucleolus. Although little differentiation between the glands can be distinguished in living material, sections demonstrate the presence of three distinct types, and these have a fairly constant distribution. The outer wall of the albumen gland is composed only of cells with granular contents which stain lightly with iron haematoxylin and mucicarmine, whereas the inner or median wall is composed of two types of cells. In one of these the small secretory spherules stain densely with iron haematoxylin and are unaffected by mucicarmine, and the second less plentiful type is mucus-secreting. These cover a narrow ventral strip of the median wall, though some may have a more scattered distribution. A layer of circular muscles is developed in the connective tissue. The albumen gland opens on to the ventral wall of the capsule gland (OAC) by a short duct lined by a columnar ciliated epithelium with a few interspersed mucous cells. The cilia are long, exceeding the height of the cells, and no subepithelial gland cells are present. The duct is surrounded by a thick layer of circular muscles, which on contraction close the passage between the two glands.

The ventral channel (Fig. 5*a*, VC), which leads posteriorly from the bursa copulatrix (Fig. 4, BC), is covered by a columnar unciliated epithelium. To the cells may be attached spermatozoa, closely packed together with their heads embedded in the distal protoplasm and their tails projecting into the lumen. The epithelium is surrounded externally by a thin inner layer of longitudinal muscles and an outer circular layer; this musculature enables sperm to be passed up the channel by peristalsis. In *Littorina* (Linke, 1933) sperms pass from the bursa copulatrix to the receptaculum seminis, which is embedded in the albumen gland, by way of two longitudinal folds. A ciliary current on the under surface of these transports the sperm balls. Longitudinal folds similarly concerned with conducting seminal fluid are described in

Viviparus bengalensis by Annandale and Sewell (1921). At the posterior end of the capsule gland the ventral channel leads into a duct (Fig. 4, DIG) which passes to the ingesting gland (Fig. 3, IG). The walls of the duct are folded longitudinally and surrounded by a very thick layer of circular muscles, and masses of sperm are invariably attached to the columnar epithelial lining. In its relationship to the longitudinal folds and in its role of harbouring sperm this region corresponds to the receptaculum seminis of *Littorina*; posteriorly, however, it opens into the ingesting gland—a brown glandular mass of tissue composed of blind tubules and having the appearance of a digestive gland. Examination of living material shows that the gland is made of only one type

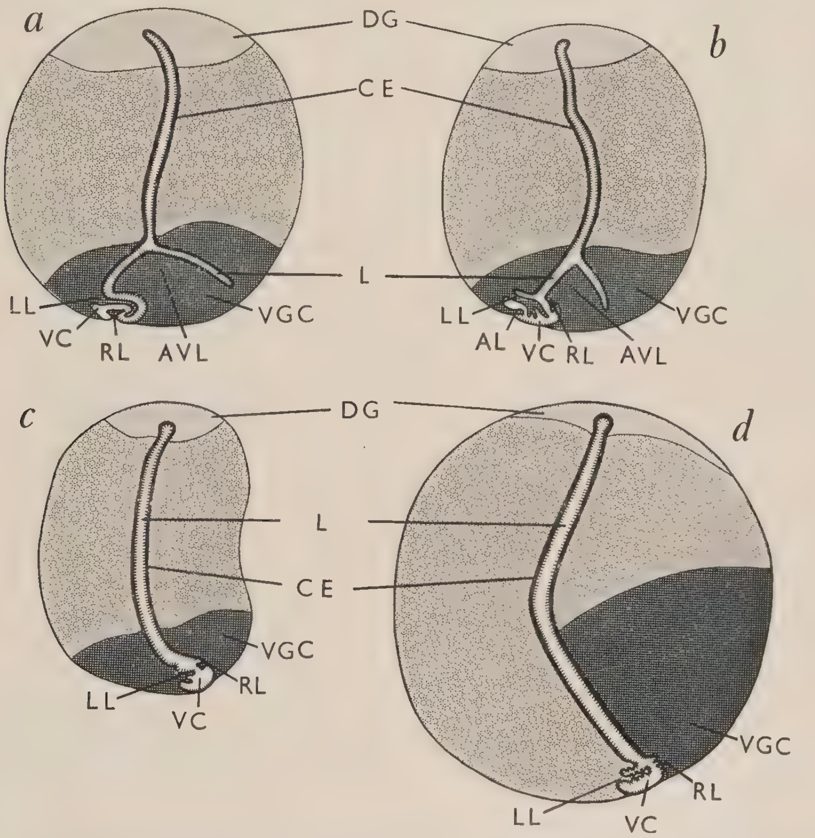

Fig. 5. Diagrammatic transverse sections through the mid region of the capsule gland.

a, *Nucella lapillus* (× 14). *b*, *Ocenebra erinacea* (× 17).
c, *Nassarius reticulatus* (× 23). *d*, *Buccinum undatum* (× 12).

The three types of stippling indicate corresponding glandular areas.

AL, accessory longitudinal fold; CE, ciliated epithelium; L, lumen of capsule gland; VC, ventral channel. Other letters as in Fig. 4.

of cell (Fig. 6*a*), which is filled with brown spherical masses (BRM) lying in vacuoles; in the lumina of the tubules large numbers of active unorientated sperm (FS) may be present, but some may lie motionless against the free edges of the epithelium as if trapped in the protoplasm of the cells (SE). In sections it is seen that the sperms are engulfed by the gland cells and lie in vacuoles in the cytoplasm (SV). Within these they are digested and it seems probable that the products or remains of digestion impart to the gland its brown colour. A layer of connective tissue, rich in blood spaces, underlies the ingesting cells and here amoebocytes (AM) are plentiful. Many of these contain brown granules which have the appearance of excretory matter, and it is probably by this means that the gland loses waste. So far as I am aware no such gland has previously been described in the Mollusca: it is, however, present in the four species of Stenoglossa dealt with here. It appears to serve as a mechanism for ridding the animal of unwanted sperm and perhaps of deriving nourishment from them.

The lateral walls of the capsule gland attain 1·75 mm. in thickness and are composed of groups of gland cells lying at various heights and packed tightly together with a very tenuous layer of connective tissue between each group (Fig. 6*b*). As in the albumen gland the ducts of the cells (DGC) run parallel with one another to open between the columnar ciliated cells which line the lumen. This ciliated epithelium covers the narrow dorsal wall, under which a thin layer of gland cells is developed (Fig. 5*a*, DG), and ventrally it spreads over the outer surfaces and free tips of the two longitudinal folds. The inner faces of these folds, forming the dorsal wall of the ventral channel (VC), are not ciliated. If the capsule gland be opened by a dorsal longitudinal incision (Fig. 4) it is seen that the right lateral wall has a ventral longitudinal cleft running from near the middle of the gland to the anterior extremity, and separating an anteroventral lobe (AVL) from the rest of this glandular area. This lobe together with two adjacent longitudinal strips of tissue—one on the right and the other on the left lateral walls—is more translucent than the surrounding areas and of a slightly yellowish hue (VGC). The lobes are composed of gland cells filled with small colourless spherules, which in sections stain lightly with iron haematoxylin and may be faintly tinged with pink by mucicarmine. After azan some spherules are reddish or orange and others blue. It would appear that two types of secretion are produced by these cells since, with azan, the final products in the lumen of the gland stain either deep orange or blue. A longitudinal area around the dorsal wall (DG) has a similar appearance in the living state. In sections of this the cells stain with iron haematoxylin and slightly with mucicarmine; after the azan stain the cytoplasm is pale blue and tiny spherules contained in it are orange in colour. With this stain the secretion is pale blue and orange, suggesting that one type of secretion is present in the cytoplasm and another in the spherules. Near the posterior end of the capsule gland two narrow transverse strips of tissue (TS), one on each side and arising near the opening of the albumen gland, separate

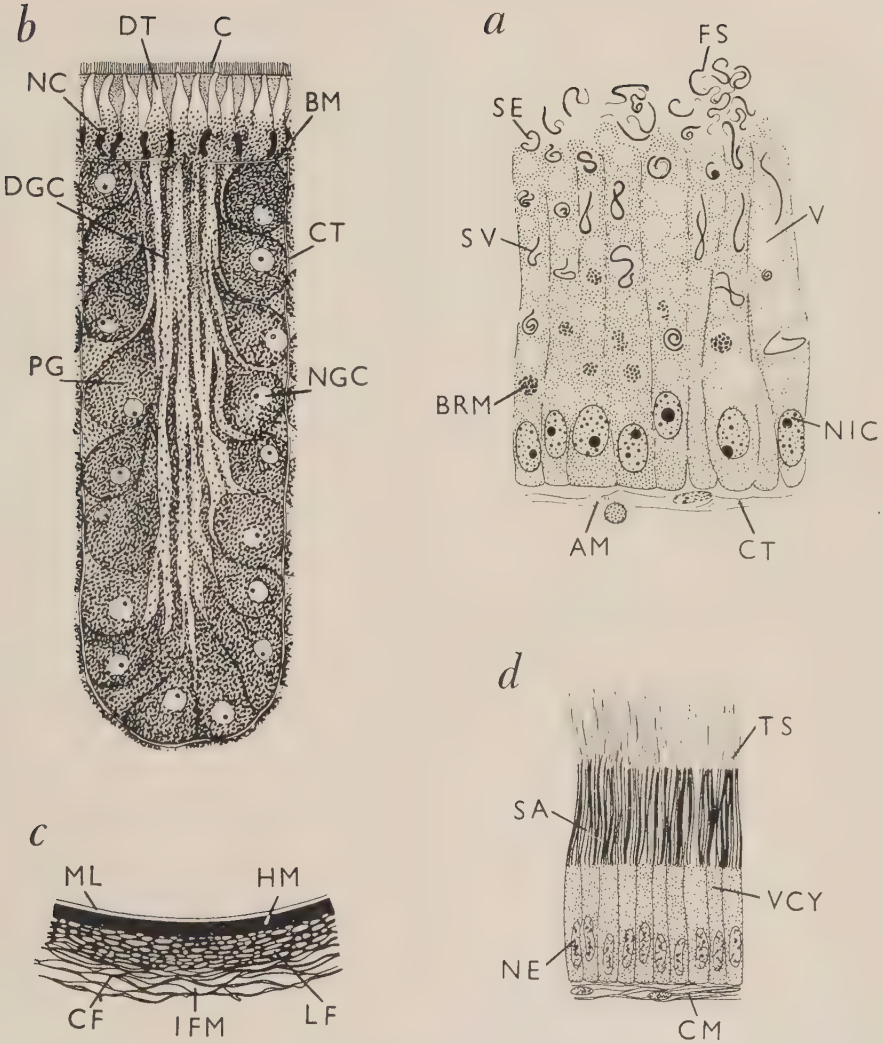

Fig. 6. *Nucella lapillus*. Parts of transverse sections through:

a, the ingesting gland, fixed in Keilin's modification of Bouin's fluid and stained in iron haematoxylin (× about 650).

b, the wall of the capsule gland, showing a single cluster of gland cells (× 200).

c, the wall of an egg capsule (× 200).

d, the bursa copulatrix, fixed in Susa and stained in iron haematoxylin (× 550).

AM, amoebocyte; BM, basement membrane; BRM, brown spherical mass; C, cilia; CF, circular fibres; CM, circular muscles; CT, connective tissue; DGC, ducts of gland cells; DT, distal tip of duct filled with mucoid protoplasm; FS, free unorientated sperm; HM, homogeneous layer; IFM, interfibrillar mucoid substance; LF, layer of longitudinal fibres; ML, inner mucous layer; NC, nucleus of ciliated cell; NE, nucleus of epithelial cell; NGC, nucleus of gland cell; NIC, nucleus of ingesting cell; PG, protein secreting granules; SA, attached sperm; SE, sperm entrapped by protoplasm; SV, sperm in vacuole of cytoplasm; TS, tails of orientated sperm; V, vacuole; VCY, vacuolated cytoplasm.

In the gland cells of *b* and in *c*, the protein secretion is shown in black and the mucoid secretion is left white.

a right and left posterior tip (PT) from the main mass of the gland. Beneath the ciliated epithelium of the strips is a layer of circular muscles and a few muscles radiate outwards; it is this musculature which distinguishes these strips of tissue and constricts them from the surrounding wall. Both they and the posterior tips of the gland are made up of mucous cells, and similar cells border the anterior extremity of each lateral lobe (MCC). The cells constituting the main part of the gland are filled with larger colourless granules which have a rather irregular oval shape (Fig. 6*b*). They stain with iron haematoxylin, but are unaffected by mucicarmine; with azan they are a deep red. The protoplasm has a different staining reaction—it is very slightly affected by mucicarmine but stains deep blue with azan. The distal tip of each duct is filled with this mucoid protoplasm (DT) and no granules are visible, a fact reminiscent of the structure of the cells of the epithelium of the mammalian stomach. Again two types of secretion are produced, one a mucoid and the other a protein. Hence all the gland cells of the capsule gland, except those of the posterior tip and anterior border of each lobe, produce a double secretion and it is the intertwining of these which is responsible for the fibrous structure of the wall of the egg capsule (Fig. 6*c*), an appearance which is obvious if secretion be taken from the gland and examined in sea water.

A layer of dense connective tissue surrounds the capsule gland and through this run muscle fibres. The musculature is best developed beneath the ventral channel and especially near the bursa. Circular muscles surround the dorsal wall and penetrate between the gland cells. Laterally, except in the posterior transverse strips, the muscles are thinly scattered—some circular muscles run below the ciliated epithelium and in the connective tissue surrounding the gland, whilst through the thickness of the wall scattered circular and oblique fibres can be traced. The vestibule, the vagina and the bursa copulatrix are all clothed by a columnar ciliated epithelium. The walls of the bursa are thrown into folds by variations in the depth of the underlying connective tissue, and sperm may everywhere be attached to the epithelium (Fig. 6*d*), whilst a mass of sperm may fill the lumen. Beneath the epithelium is a thick muscular coat, consisting of an outer circular layer with longitudinal and oblique bands beneath the folds. Among the ciliated cells covering the vestibule are a few gland cells filled with spherules which stain black with iron haematoxylin and red with azan. The vestibule and vagina are extremely distensible; the walls are folded longitudinally and the arrangement of the layers of muscle is similar to that of the bursa.

The eggs are liberated from the oviduct by the relaxation of the sphincter at the entrance to the albumen gland. Here they come under the influence of ciliary currents (Fig. 4). The opposing currents drive the secretion from the epithelium and whirl it into the lumen mixing it with the eggs, and at the same time the whole mass is propelled along the gland. It then passes through the short duct which leads into the capsule gland (OAC), being drawn through by the relaxation of the circular muscles and by the action of cilia. In the capsule gland the forward movement of the mass of eggs is arrested whilst the wall of the capsule is secreted around it. The ciliary currents on the wall of the gland are weak. Mucus secreted by the posterior tips is directed forwards; but anterior to the tips the currents beat at right angles to the long axis of the gland—in some places away from, in others towards, the ventral channel. The currents mix and distribute the various secretions from the gland cells immediately they are poured forth into the lumen. At the anterior end of the gland the currents are directed towards the vagina (VA) and on the longitudinal folds of the ventral channel (LL, RL) they beat outwards. These folds are usually hidden from view by the overhanging glandular lobes. The passage of the egg capsule through the vestibule (V) is effected by muscular means assisted by the action of cilia beating towards the genital aperture.

Ocenebra erinacea

The female genital system of this stenoglossan is built on the same plan as that of *Nucella*. There are, however, the following minor differences in morphology and histology.

The gonopericardial duct appears to be wider and it opens into the pericardium by a very prominent ciliated funnel: so wide is the duct, in fact, that on relaxation of the circular muscles a mature egg may enter it. Only two types of subepithelial gland cells have been observed in the albumen gland. The more plentiful type is filled with spherules which are colourless in life, stain lightly with iron haematoxylin and are a very faint pink after mucicarmine. These cells cover the outer wall in the main part of the gland and spread on to the inner wall, where they are replaced by mucous cells, which in the posterior part of the gland, near the opening of the oviduct, completely encircle the columnar ciliated epithelium. The duct from the albumen gland opens into the ventral channel, and receives near this opening the duct from the ingesting gland. In this gland there is resorption of yolk granules as well as ingestion of spermatozoa. It is possible that these yolk granules have been derived from eggs which for some reason failed to be included in a capsule. This may also occur in *Nucella*, but has never actually been observed. The ventral channel (Fig. 5*b*, VC), which is formed between the two longitudinal folds of the ventral wall of the capsule gland, is subdivided into two regions by a third longitudinal fold of tissue (AL). This arises soon after the channel emerges from the bursa and extends to the opening of the albumen gland. It lies beneath the left longitudinal fold, which completely envelops it, and in the channel formed between them the epithelium is not ciliated. Ciliated cells cover the channel between this third fold, which is not represented in *Nucella*, and the outer or right longitudinal fold. The albumen gland opens into the ciliated region and the transference of sperm is confined to the unciliated part.

The capsule gland is divided into the same regions as in *Nucella*, but there are slight variations in the types of gland cells composing the dorsal (DG) and ventral (VGC) longitudinal strips and the posterior tips of the thick lateral glandular lobes. The transverse strips of tissue which arise near the opening of the albumen gland, and are constricted off from the surrounding glandular areas, are made up of mucous cells. Posteriorly these are replaced by mucoid cells which often have a pink tinge. In the anteroventral lobe (AVL), separated from the right lateral lobe by a deep cleft, and in the two adjacent longitudinal strips of tissue, the cells (VGC) stain blue with azan and some spherules are reddish. These cells probably produce two types of secretion, but this is less obvious than in *Nucella*. The secretion in the lumen of the gland stains blue with azan and small isolated patches may be reddish or orange. The longitudinal glandular strips surrounding the narrow dorsal wall (DG) are composed for the greater part of mucoid cells, but these produce only one type of secretion and together with the anterior border of each lateral lobe stain purple when the whole gland is opened and placed in thionin. There is, however, a narrow longitudinal band of cells on each side between this dorsal strip of mucoid cells and the main part of the capsule gland which elaborates a second type of secretion, the spherules within the cells and the secretion staining bright orange with azan.

The histology of the bursa copulatrix is of some interest. If this pouch be cut open it may be seen that the white glandular walls are folded transversely and have a velvety appearance. The epithelium is composed of one type of cell, which is elongated, and the free edge is produced into a large number of stiff processes each equal to about a quarter of the total length of the cell. In the proximal half of the epithelium the cytoplasm is vacuolated, and the vacuoles contain small spherules which stain lightly with iron haematoxylin and red with azan. Distally the cytoplasm contains a mucoid substance into which the spherules migrate. The secretion in the lumen of the bursa consists of irregularly shaped mucoid masses with numerous spherules, and sperms introduced into it collect around this secretion. The nuclei of the cells of the epithelium, each with one or two nucleoli, are round or oval in outline. They lie towards the base of the cytoplasm, but migrate to the free tips of the cells when secretion is being liberated.

The musculature and the ciliary currents of the genital duct are practically identical with those described for *Nucella*. Here again the importance of the ciliary currents of the capsule gland appears to be in mixing and distributing the different types of secretion to form the wall of the egg case.

Nassarius reticulatus

Nassarius reticulatus resembles *Nucella* in the main points of the structure of this system, but differs from it in some details; these alone will be dealt with in the following paragraphs.

From the anterior end of the oviduct, near which the gonopericardial duct is given off, the laterally compressed albumen gland widens into a comparatively deep pouch, its dorsal wall being deeply convex and the ventral wall concave. The ciliary currents on the wall of the gland are similar to those in *Nucella*. The subepithelial glands are of two types only: mucous cells beneath the inner wall and mucoid cells beneath the outer. The albumen gland opens into the ventral channel of the capsule gland and near this opening receives dorsally the duct from the ingesting gland. The duct is longer than in *Nucella* and is somewhat coiled. The gland is less voluminous and amongst the ingesting cells which line the tubules are mucous cells. The posterior tips of the capsule gland may be brownish in colour or may have a rather transparent appearance. They are composed of mucous cells. Anteriorly each lateral lobe of the gland terminates in a reddish glandular area—during life the gland cells are filled with small orange granules. These stain deeply with iron haematoxylin and after azan the granules and the final secretion are red and the cytoplasm around the spherical basal nuclei pale blue. A transverse strip of mucous cells separates this area from the more posterior part of the gland. The right lobe is not subdivided (Fig. 5 *c*), and the longitudinal strips of gland cells (VGC), which border the anterior two-thirds of the ventral channel (VC) on each side and surround the dorsal end of the lumen (DG), do not produce a double secretion. The ventral strips are composed of mucoid cells and the dorsal region of mucous cells. The secreting cells, which constitute the main part of the capsule gland, contain granules of the same shape as the corresponding cells of *Nucella*, but they are smaller. They stain deeply with iron haematoxylin and bright orange with azan. Their secretion in the lumen of the gland has a similar staining reaction. The arrangement of the ciliary tracts on the walls of the capsule gland differs slightly from that of *Nucella*. The transverse currents are confined to the upper half of the gland, whilst anteriorly the currents run towards the exterior.

The ventral channel is ciliated throughout and the longitudinal folds which form its dorsal wall (LL, RL) are less pronounced than in the molluscs already described. Anteriorly the capsule gland and the ventral channel lead to the muscular vestibule which is lined by a columnar ciliated epithelium. Mucous cells are interspersed among the ciliated cells and some mucous glands lie in the connective tissue beneath the epithelium. The walls of the vestibule are thrown into deep folds. Muscles radiate from the folds to longitudinal and circular muscles which surround the whole structure. The longitudinal folds of the ventral channel arise at the anterior end of the vestibule beneath the opening of the bursa.

If the mantle cavity of *Nassarius* be opened the genital aperture is immediately obvious, and through it, since it is of large diameter, can be seen two further openings, one dorsal and the other ventral. The dorsal one is the aperture of the bursa copulatrix and the ventral leads to the vestibule of the oviduct. The space between the genital opening and these two inner ones thus represents the vagina. This and the bursa are covered by a columnar ciliated epithelium with an ample supply of mucous cells. The walls of the bursa are folded and beneath the epithelium is a thick muscular coat consisting of circular, oblique and longitudinal fibres.

Buccinum undatum

The gross anatomy of the female genital system of *Buccinum undatum* was described by Dakin (1912), but many details in its structure were not observed. Of these the most outstanding are the bursa copulatrix, the ingesting and resorptive gland, and the gonopericardial duct. The general anatomy of the genital duct conforms to the typical stenoglossan plan and in many respects it approaches most closely to *Nassarius reticulatus*. The albumen gland assumes similar proportions. From the thin-walled oviduct it passes dorsally and enlarges to form a pouch, which is laterally compressed and opens anteriorly into the capsule gland by a wide aperture. The dorsal wall of the pouch is deeply convex and the ventral wall concave. Two types of gland cells are present—mucous cells constitute the thickness of the inner wall and spread on to the outer wall of the gland. Here, except near the opening into the oviduct, they are replaced by cells containing small spherules embedded in the vacuolated protoplasm. The spherules stain deeply with iron haematoxylin and blue with azan; after mucicarmine the protoplasm is faintly pink.

The duct from the ingesting gland opens into the dorsal wall of the genital duct at the junction of the albumen and capsule glands. The walls of the duct are deeply folded and during the breeding season numbers of orientated sperm are attached to the columnar ciliated epithelium. A thick muscular coat which surrounds the walls is chiefly composed of circular fibres, and radial muscles penetrate the folds. The duct opens into the glandular tubules, in which masses of yolk granules and unorientated spermatozoa have been observed during the months of April and May. As in *Ocenebra* the yolk granules and the sperm are taken up by the absorbing cells and apparently digested.

On the lateral walls of the albumen gland the cilia beat away from the narrow dorsal and ventral walls and the current is directed downwards and slightly backwards from the duct of the ingesting gland. Around the opening of the capsule gland the cilia beat posteriorly. The effect of these currents is to mix albuminous secretion with the eggs from the oviduct and probably to direct sperm from the duct of the ingesting gland and from the ventral channel on to the egg mass. Meanwhile the contents of the albumen gland are prevented from passing into the capsule gland. The musculature of the albumen gland is well developed: circular muscles lie beneath the ciliated epithelium and they are also scattered between the groups of gland cells and in the connective tissue surrounding the gland.

The right lobe of the capsule gland (Fig. 5*d*) is not subdivided as in *Nucella* and *Ocenebra*. After the breeding season the glandular tissue is greatly reduced and the lateral walls may be thrown into transverse folds. The posterior tips of the gland are composed of mucous cells, and similar cells underlie the dorsal wall (DG), where they form a narrow longitudinal strip. Anteriorly each lateral lobe is bordered by mucous cells, and mucoid cells constitute the ventral half of the anterior two-thirds of the right lobe (VGC). In living tissue these areas can be distinguished by their slightly transparent appearance. The main mass of the gland is opaque white and is composed of one type of cell which is filled with small spherules staining deeply with iron haematoxylin and azocarmine. The ciliary currents on the walls of the capsule gland are similar to those of *Nassarius*.

The longitudinal folds which separate the ventral channel from the lumen of the capsule gland are less pronounced than in *Nucella*. The left fold (LL) is obvious on dissection and the ciliated epithelium which covers it, and the wall immediately dorsal, is thrown into longitudinal undulation by variations in the height of the cells and in the thickness of the underlying connective tissue. The right fold (RL) is represented by a number of similar though more pronounced undulations in the ciliated epithelium. Only a narrow strip of cells on the wall of the ventral channel (VC) is unciliated. Mucous cells are interspersed among the columnar cells, especially at the anterior end, and they also occur in the connective tissue beneath the epithelium. Posteriorly in the region of the transverse muscular strips the longitudinal folds terminate and the ventral channel divides into two grooves. One passes to the right and runs dorsally between the albumen and capsule glands to the duct of the resorptive and ingesting gland, and the other takes a similar course on the right side. The ciliary currents on their walls are directed towards the gland.

The muscular vestibule into which the capsule gland opens is covered by a columnar ciliated epithelium. The walls are longitudinally folded. Radial muscles are developed in the connective tissue beneath the folds and these are surrounded by a layer of circular muscles which increases in thickness towards the genital opening. The longitudinal folds of the ventral channel arise near the mid region of the vestibule and in the epithelium which covers them, and in the underlying connective tissue mucous cells are plentiful. Anterior to the origin of the folds mucous cells are distributed throughout the epithelium. Here the lumen of the vestibule is approximately circular in transverse section with a diameter of 0·5 mm., and is surrounded by a layer of circular muscles 1·25 mm. in thickness. The vestibule and the bursa copulatrix open into the vagina. The bursa is a large and very muscular sac, dorsal in position. The walls are deeply folded and covered by a columnar unciliated epithelium which contains yellow pigment granules. Amoebocytes are found between the epithelial cells and in the lumen: they appear to act as scavengers and carry off any available prostatic secretion. Although the longitudinal folds of the ventral channel do not extend to the opening of the bursa the ventral groove runs to this point. Spermatozoa are passed from the dorsal opening of the bursa into the ventral groove and conveyed up the ventral channel of the capsule gland. The channel is surrounded by circular muscles and some longitudinal fibres, and the conduction of sperm is probably assisted by peristalsis, as in *Nucella*, though this has not been observed in *Buccinum*. The short vagina is lined by a ciliated epithelium in which no mucous cells occur. It is surrounded by a sphincter which guards the genital aperture.

THE VENTRAL PEDAL GLANDS

The female *Ocenebra erinacea* has two ventral pedal glands. In anaesthetized animals the anterior one may sometimes be seen as a conical papilla, with a small central cavity, projecting from the surface of the foot. It lies a short distance behind the anterior pedal or pedal mucous gland in the mid line. Usually the gland is not visible. The epithelium of the papilla is very high, attaining 180μ, and is composed of ciliated cells and gland cells. The ciliated cells are narrow at the base and broaden distally; their nuclei are elongated and lie towards the upper half of the cytoplasm. The gland cells arise from the basement membrane to alternate with the ciliated cells. On fixation these glands tend to empty their secretion, leaving within the cells a granular lightly staining cytoplasm. The secretion, often entangled in the short thick layer of cilia, is black after iron haematoxylin. A second, comparatively infrequent type of gland cell is distributed in the connective tissue below the summit of the circular fold—that is around the aperture leading to the central cavity of the papilla—and in the epithelium. It contains spherules which stain deeply with azocarmine and rather lightly with iron haematoxylin. The lips are covered by a columnar ciliated epithelium, which is very low on their inner surfaces. Among the ciliated cells are a few gland cells similar to the second type of gland cell described in the gland, and these are also found in the epithelium covering the surrounding area of the foot.

The second ventral pedal gland is a short distance behind the first. It has the appearance of a pit, which in fixed material measures 2 mm. deep and 0·6 mm. broad and has thick glandular walls thrown into folds parallel to the surface of the foot. The lumen in transverse section is a crescentic slit with a concave anterior surface and a convex posterior surface. The pit is lined by a columnar ciliated epithelium and gland cells are scattered among the ciliated cells. These have basal nuclei and the cytoplasm is filled with small protein secretion granules. Below the epithelium is a dense layer of mucous cells grouped in clusters and in the connective tissue which separates one cluster from its neighbours are developed radial muscles. Circular and longitudinal muscles lie beneath the basement membrane of the epithelium and the ducts of the mucous cells pass between them to open into the lumen of the gland.

In the male there is a single ventral pedal gland identical in structure with the anterior one of the female.

Two ventral pedal glands are present in the female *Nucella lapillus*. These are comparable in position to the two glands described in female *Ocenebra*. In both animals the structure of the anterior gland is similar, but in *Nucella* it has not been seen to project from the surface of the foot, though the musculature suggests that this is possible. It communicates with the exterior by way of a ciliated duct, which has an inconspicuous external aperture lying between the circular opening of the posterior and that of the anterior pedal gland. In

Nucella the posterior gland has the form of a deep conical pit, the walls of which may be thrown into folds running along the long axis of the walls. It is lined by a columnar ciliated epithelium, and among the ciliated cells are a few gland cells with spherules which tend to dissolve on fixation. The cytoplasm stains red with azan and lightly with iron haematoxylin. Beneath the basement membrane of the epithelium is a layer of circular and longitudinal muscles through which pass the ducts of underlying gland cells. These cells form a thinner layer than the corresponding cells in *Ocenebra*: in sections their cytoplasm is vacuolated and the contents of the vacuoles dissolved. The protoplasm stains blue with azan and lightly with iron haematoxylin. It is unaffected by mucicarmine or toluidin blue but the final secretion attached to the cilia and the surface of the epithelium stains lightly with these stains for mucus. Radial muscles penetrate the connective tissue of the folds and pass between the groups of subepithelial gland cells.

In the male only the anterior gland is present.

In male specimens of *Nassarius reticulatus* no pedal gland has been observed, but one is present in the female. It opens in the mid-ventral region of the foot a short distance behind the anterior pedal gland and resembles the posterior pedal gland of *Ocenebra* and *Nucella*. The gland forms a deep but narrow pit, broad laterally and narrow from back to front. At the dorsal end it is rounded. The walls are folded transversely and are covered by a columnar ciliated epithelium. Beneath the basement membrane of this epithelium is a layer of muscles: most of the fibres are longitudinal with respect to the pouch, but a few are circular. A layer of gland cells underlies the muscles, the cells being grouped in small clusters and their ducts passing through the muscle layer and between the ciliated cells to discharge their secretion into the lumen of the gland. After fixation in Susa the cytoplasm of the cells appears highly vacuolated, although the contents of the vacuoles have dissolved. The protoplasm stains blue with azan and lightly after iron haematoxylin; but it is not affected by mucicarmine, although the secretion in the lumen of the gland and that in some of the ducts is stained pink. It thus appears that the cells produce a mucous secretion, the precursor of the secretion not being affected by the recognized stains for mucus. Radial muscles penetrate the folds of the wall and make their way between the groups of gland cells.

The presence of a ventral pedal gland in *Buccinum undatum* has been denied by the majority of previous investigators. Carrière (1882) failed to find one and his results were adopted by Simroth (1907). In 1910 Pelseneer confirmed Carrière's observations for both sexes, stating that the gland was present only in the embryo. Dakin (1912) described the anterior pedal gland (= the pedal mucous gland) and apparently failed to discover the presence of a ventral one. He obviously confused the anterior gland with the ventral and held that the former was homologous with the pedal pore, or opening of the ventral pedal gland, of other prosobranchs, which had once been considered as the external opening of a water vascular system. He mentioned, without any reference, that in *Purpura* (= *Nucella*) Pelseneer had ascribed to the *anterior* gland the function of secreting the egg capsule and concluded that in *Buccinum* it did the same. But in 1910—before the publication of Dakin's monograph—Pelseneer had figured both an anterior and a ventral pedal gland in *Nucella*, showing the egg capsule in the *ventral* one and had explicitly stated that the capsule was formed there. In a note to *Nature* Cunningham (1899) described egg capsules in the "sole gland" or ventral pedal gland of *Buccinum*, and so far as I know this is the only occasion on which the presence of a ventral pedal gland has been noted in this mollusc. This gland, too, was mistaken by Dakin for the anterior gland. Observations on living animals show that in male specimens there is nothing to correspond to the ventral pedal gland, but in females it can be seen as a shallow pouch some distance behind the anterior gland in the mid line.

The columnar epithelium covering the pouch is ciliated and gland cells are distributed throughout the ciliated cells. In one type of gland, which is very plentiful, the cytoplasm contains small spherules staining red or orange with azan and with varying degrees of intensity with iron haematoxylin; occasionally the spherules stain blue with azan. Mucous cells are numerous in the epithelium, and in the connective tissue below there are small irregularly shaped gland cells with contents staining deeply with iron haematoxylin; their narrow ducts penetrate the basement membrane to open between the epithelial cells. The walls of the pouch are deeply folded by variations in the thickness of the underlying connective tissue and musculature of the foot. Radial muscles penetrate the folds and external to these is a coat of circular fibres.

The whole pouch is covered with an epithelium of the same height and general histological character as is found on the neighbouring area of the foot; in the gland, however, mucous cells are fewer and the other types of gland cell are more numerous.

THE EGG CAPSULES

The egg capsule of *Nucella lapillus* is an erect vase-shaped structure about 8 mm. high. It is circular in transverse section and broadest in the middle where its diameter is about 2 mm. At one end the capsule tapers to form a short stalk which attaches it to a basal disc, and the disc is firmly anchored by cement to the substratum. At the opposite end there is a circular aperture which is filled with a plug of a rather transparent homogeneous substance. Two longitudinal lines of thickening can be traced over the smooth surface, and these are placed so as to divide the wall into two approximately equal halves. Distally the suture of one side meets that of the other over the surface of the plug, which is thus subdivided. The minute structure of the wall has been described by Ankel (1937) who states that it consists of three layers: an inner layer of a homogeneous transparent substance, and middle and outer layers of fibres orientated in definite ways and separated from one another by distinct spaces. He described the inner fibres as running in a longitudinal direction and the outer fibres as being circular. Transverse sections through the wall of the capsule of *Nucella* (Fig. 6*c*) show this fibrillar appearance clearly and, if the sections be stained with azan, the three regions may be readily distinguished: the inner homogeneous layer (HM) stains red, and in the fibrous layers (LF, CF) the fibres are red, whilst the interfibrillar spaces are filled with a blue staining substance (IFM). Externally the fibrillae are more widely separated by the interfibrillar substance and are thinner. In sections stained with iron haematoxylin the inner homogeneous layer stains deeply and the fibres of the outer layers more lightly. The interfibrillar substance is hardly affected by this stain but is pink after mucicarmine. These results suggest that the wall of the capsule is composed of two substances—a protein which according to Ankel is conchiolin, and, intermixed with the protein in the outer layers, a mucous or mucoid substance. It is the distribution of these two substances which gives the fibrillar appearance to the capsule. When the capsule has weathered for some time the mucus tends to contract leaving the spaces between the fibres observed by Ankel. In the stem and in the basal disc the fibres are more irregularly disposed. The plug is made of mucus and is tightly fitted into the distal aperture of the capsule. A very thin mucous layer also covers the wall internally (ML) and is sparsely distributed over the outer surface. The capsule contains several hundred eggs embedded in an albuminous fluid, but of these only about 15–25 hatch, the remainder being devoured by their fellows (Lebour, 1937).

The egg capsule of *Ocenebra erinacea* is built on the same plan as that of *Nucella*. It is about the same height and a narrow stalk connects it to a basal disc by which it is fixed to the substratum. The capsule is flattened on one surface and is rounded on the other, and the middle of the rounded surface may be produced into a slight keel. In transverse section it is therefore triangular. At the distal end is a circular hole blocked by a mucoid plug. Two longitudinal sutures are visible on the smooth yellowish wall, running up the centre of the convex and concave faces and continuing over the plug. Sections stained in azan show that the microscopic structure of the wall is the same as that of *Nucella*, the inner homogeneous layer occupying a third of the total thickness and the fibrous coats the outer two-thirds. The capsule contains from 12–20 eggs embedded in an albuminous fluid and all may develop into young tingles (Lebour, 1937).

The laying of the egg capsule of *Nassarius reticulatus* has been described by Ankel (1929), and he points out the superficial resemblance between the capsule passed out from the oviduct and its more familiar form attached to the rocks or stones of the seashore. The fully formed capsule is fixed by a basal disc from which it enlarges into a vase-shaped structure slightly convex on one surface and flattened on the other. It is about 5 mm. high and about 4 mm. across at the broadest point. The walls are thinner and more transparent than in the capsules of either *Nucella* or *Ocenebra*. Examination under a binocular microscope shows that they have a fibrillar structure. The lateral margins of the flattened sides of the capsule are continued out to form a flange on each side, and this is extended over the distal end by a similar flattening of the plug. When the capsule leaves the oviduct the walls have a more opaque appearance than they have in their final state, and there are two longitudinal lines of thickening containing the material for the flanges.

Sections of capsules taken from the vestibule of the oviduct show that the wall is composed of several layers staining blue, red or orange with azan. The inner layer is a mixture of two secretions one of which stains orange and the other blue; the next is a homogeneous layer of orange-staining material and the third resembles the first. Along the longitudinal lines of thickening there is an increase in both types of secretion and the layering is ill defined. Covering the outside is a very tenuous coat of secretion which stains a bright red. Capsules collected from the shore and similarly treated show a surprisingly different structure: the wall appears to be thinner and, except in the region of the flanges, is remarkably homogeneous. It stains orange with azan, but here and there a trace of the blue secretion can be found, mainly confined to the core of the flanges. These differences between the two capsules are due to the moulding action of the ventral pedal gland.

Each capsule contains an albuminous fluid in which float up to a hundred eggs, all of which may develop into free living larvae (Lebour, 1937; Portmann, 1925).

The egg capsules of *Buccinum undatum* are laid in large masses; the lower ones of each mass are fixed to the substratum and the upper ones are piled upon them and attached to them by their bases. The bases are oval and the edges project to form flanges by which the attachment is effected. Each capsule is compressed, one side being flattened and the other convex. It is broadest at the base, which measures 12 mm. and is about 10 mm. in height. The flattened face is smooth, and towards the distal edge, in a subterminal position, is the oval plug, of a different appearance and consistency from the rest of the wall. The convex face is covered by numerous wrinkles which form a reticulate pattern over its surface, and the half of the flange which projects from the base of this face is similarly ridged. From each side of the oval plug a line of thickening passes to the flattened edge, the two lines representing the sutures which, in *Nucella*, divide the capsule into equal halves.

The structure of the wall resembles that of the capsules already described. In section it appears to be made up of four distinct layers compressed together. On the outside is a homogeneous covering and through it can be seen a coat of circular fibres, both these layers staining deeply with azocarmine and iron haematoxylin: the fibres are separated by a mucous secretion. Next comes a layer of similar but broader longitudinal fibres embedded in a mucoid secretion, and on the inside is a thin mucous sheet. The outer covering is spread over the surface of the plug, which, together with the base, is composed chiefly of mucus.

The egg case contains several hundred eggs embedded in albumen; out of these only about ten come to maturity, the rest being used as food (Portmann, 1925).

THE FUNCTION OF THE FEMALE GENITAL DUCT AND OF THE VENTRAL PEDAL GLAND

Nucella lapillus

At the time of copulation the male passes the penis through the female genital aperture so that the tip is directed into the bursa copulatrix. Spermatozoa are released into this pouch and attach themselves to the epithelial cells by their heads; these orientated sperms lie closely packed side by side with their tails pointing into the lumen of the bursa, in which unorientated sperm may also lie. After copulation the spermatozoa may be passed up the ventral channel of the uterus by peristalsis, assisted perhaps by their own activity, and they may become attached to the epithelium of the channel or of the duct of the ingesting gland. It is not known how soon after copulation the formation of the egg capsules begins, but evidence suggests that at least on some occasions the two events follow closely upon one another. Several specimens of *Nucella* will often be found associated with a batch of egg capsules: some of these are usually males and others females, and whilst some females may be copulating others will be laying eggs.

The method by which the capsule is manufactured by the genital duct may be partly deduced from the observations given above and verified by the study of living animals. A large number of eggs are passed down the oviduct into the albumen gland and here they are mixed by ciliary activity with the albuminous secretion. At such a time the walls of the gland are greatly distended by the mass of eggs. The exact locus of fertilization is uncertain: if sperms from the ventral channel and the duct of the ingesting gland are passed into the albumen gland on the relaxation of the sphincter muscle which guards its opening, then fertilization will occur in the lumen of this gland. Otherwise spermatozoa may be poured on to the eggs as they enter the capsule gland.

The mass of albumen and eggs is passed into the capsule gland by muscular assisted by ciliary action, and here the process of capsule formation begins. Only on one occasion has a capsule been found within the gland, although numerous specimens have been examined in the hope of finding capsules in various stages of formation. In this case the capsule was near completion and occupied the whole length of the gland, the plug being at the upper end, near the posterior mucous tips, and the base anterior. The longitudinal sutures which divide the capsule into two halves were lying one against the ventral channel and the other against the narrow dorsal wall. The wall of the capsule was not so smooth as in its final form, nor was it so completely circular in transverse section. The longitudinal sutures were more obvious and the basal disc was represented by a rather irregular fibrous mass. From the orientation of this capsule may be deduced the functions of the various regions of the gland. Since the plug is composed of mucus it is apparently secreted by the posterior mucous tips and the capsule wall by the rest of the gland. As already stated the wall of the capsule is made of three layers: the innermost is composed of a homogeneous substance which stains an orange-red with azan and black with iron haematoxylin, is similar to the more plentiful secretion from the cells of the main part of the capsule gland, and also resembles the non-mucoid secretion from the cells of the dorsal wall and those which border the greater part of the ventral channel—it is therefore presumably derived from them. Outside this layer is a double fibrous coat formed by intermingling this same protein secretion with a mucoid substance. The way in which this occurs and the manner of formation of the egg case as a whole may be reconstructed as follows.

Prior to the entry of the secretion and eggs from the albumen gland the cavity of the capsule gland becomes filled with a mass of secretion from the gland cells in its walls. These secrete first the protein matter and then, in increasing quantities, the mucoid substance which they produce, and the result is that the lumen is filled with a mass of secretion which is almost pure protein in the centre but consists, in its outer layers, of an emulsion in which the mucoid material acts as a dispersed phase in a continuous phase of protein. Because of the steadily increasing quantity of mucus and of the accompanying decrease in the production of protein the size of the mucoid droplets steadily increases from the centre to the periphery of the mass, and the strands of protein which separate the drops gradually decrease in size. The predominantly transverse direction of the ciliary currents on the walls of the gland rotates the mass and draws out the drops of mucoid material into streaks parallel to the transverse axis of the gland, and the protein material separating them is drawn out into strands elongated in the same direction. When the duct between the albumen and the capsule glands is opened, the albumen and the eggs are forcibly passed into the central portions of the secretion lying in the latter, so as to invaginate this into a vase-shaped structure with a round hole at the inner end into which the plug will later be fitted. This process of invagination deposits the eggs and their accompanying albumen in the centre of the mass of secretion occupying the capsule gland, which is composed of unmixed protein material, and from this is formed the innermost layer of the capsule. It has also the effect of drawing the outer emulsion of protein and mucoid secretion into sheets around the inner homogeneous layer, in which the direction of elongation of the mucoid droplets now lies parallel to the direction of movement of the eggs, that is, parallel to the long axis of the gland: from this results the longitudinal direction of the strands of the inner part of the fibrous coat. The outer part of this layer, being still exposed to the ciliary currents on the wall of the gland, retains the original alignment of the drops and so gives rise to the outer part of the fibrous coat in which the fibres are circular in direction. With the disappearance, on exposure, of the mucous dispersed phase the space occupied by it is left as a series of lacunae separating what now appear as strands of fibrous material. The mucus which lines the wall internally is secretion from the posterior tips, which was dragged along with the mass of eggs when the latter passed into the capsule gland. These tips continue to pour out secretion while the wall of the capsule is being elaborated by the more anterior parts of the genital duct. The mucus forms an accumulation which is fitted into the hole in the upper part of the capsule by the muscular action of the transverse strips which border the mucous tips anteriorly. These press the upper edge of the wall of the capsule on to the mass of mucus so that the cavity within is securely closed. The suture which divides the plug into two equal halves demarcates the limit of the secretion produced by each posterior tip.

The egg capsules are passed from the oviduct to the posterior ventral pedal gland along a groove situated in the right anterior portion of the foot and temporarily formed for that purpose. When examined in this position, it can be seen that the gland fits tightly over the capsule, and only the tip of the basal disc is protruded from the sole of the foot. The wall of the capsule is compressed and moulded to the final, smooth, vase-shaped outline. The stalk is constricted from the basal disc and the latter finally pressed out and fixed to the substratum. The mucous secretion from the subepithelial cells of the gland acts as a lubricant during the fashioning of the egg case. Secretion from the second type of gland cell may have a similar function, or may, in some way, be concerned with the hardening of the wall.

The anterior ventral pedal gland is equally developed in both sexes. Its function is obscure. Since, however, a corresponding gland is present in *Ocenebra erinacea* and both animals are carnivorous feeders, obtaining their food by boring the shells of other molluscs, it is possible that the gland may be concerned with their specialized feeding mechanism (Graham, 1941).

Ocenebra erinacea

Since the structure of the female genital duct of this mollusc and of the egg capsules produced by it so closely resembles those of *Nucella lapillus*, it may be concluded that the mode of functioning of the system is similar in the two animals.

The shape of the completed egg capsule of *Ocenebra* differs from that of *Nucella*, and this is due to a difference in the shape of the ventral pedal gland which moulds it. In the former the capsule is much more drastically changed by this gland than in *Nucella*. In the final state the two longitudinal sutures which are formed in the genital duct are situated one in the middle of the concave surface and the other in the middle of the convex. From this it may be deduced that the orientation of the capsules in the pedal gland must always be the same.

Nassarius reticulatus

The functioning of the female genital ducts of *Nassarius* agrees with that of *Nucella*, but there are some differences in the structure of the capsule which need explanation. When the capsule is being formed it does not occupy the whole length of the capsule gland, with the effect that as it passes outwards successive layers of secretion are poured over it. The plug is formed by the posterior mucous tips and the wall is moulded around the plug by the transverse muscular strips. Anterior to these the ciliary currents beat at right angles to the long axis of the gland, and they mix the mucous and mucoid secretions from the cells overlying the dorsal wall and bordering the ventral channel with that from the rest of the gland. In this way the inner layer of the capsule wall is formed. Over the anterior part of the gland the ciliary currents are directed forwards and there is little mixing of the secretion. That produced by the mucous or mucoid secreting cells is mainly confined to the dorsal and ventral lines of thickening, whilst the homogeneous secretion from the rest of the gland forms the second layer of the wall. Mucus is then mixed with this secretion as the capsule is passed to the anterior tips of the gland, since these are bordered posteriorly by a strip of mucous cells. Finally the anterior tips coat the wall with yet another thin layer which stains deeply with azocarmine and iron haematoxylin. The muscular vestibule then expands to receive the capsule and it is passed to the genital aperture. At this stage the case is biconvex and two longitudinal lines of thickening extend from the plug to the thick basal plate. The capsule is directed to the ventral pedal gland along a groove in the foot, and here a mucoid secretion is poured upon it which may act not only as a lubricant but as a hardening mixture. The walls are compressed and the flanges are formed from the longitudinal lines of thickening. At the same time the basal disc is constricted from the capsule, which assumes its final flattened shape in correspondence with that of the lumen of the pedal gland.

Buccinum undatum

Some knowledge of the functioning of the female ducts of *Buccinum* has been obtained by studying specimens during the process of egg laying and also by dissecting such animals. Unfortunately only one capsule, and that fully formed, was found still within the oviduct. This differed in several respects from the finished cases which are fastened to the rocks on the shore. The capsule was broadly oval in shape and its surface was smooth; the plug occupied an approximately terminal position and the fins around the base were thicker. Microscopically the walls were more loosely compacted.

On leaving the genital duct such a capsule is passed along a temporary groove of the foot, which runs from near the genital aperture to the ventral pedal gland. Within the latter it is retained for several minutes and is meanwhile kneaded by the pedal musculature which flattens the capsule and stamps the reticulate pattern on to the convex face. The flanges are compressed and anchored to the substratum.

The manner in which the four layers making up the wall of the capsule are laid down around the mass of eggs appears to be as follows. The three inner layers are similar to the layers of the capsule wall in *Nucella* and are formed in a corresponding manner except that the secretion is derived from the upper part of the gland only—the capsule occupying only about half the total length of the gland during its formation. Whilst this secretion is taking place a mass of mucoid and protein material is produced from the walls of the whole lower section of the gland. On the completion of the formation of the wall of the capsule in the upper half, the capsule is pushed against this material, which is thus collected into a heap situated at the base of the capsule—in this way the material for the basal disc and its flanges is brought into its correct position in relation to the rest of the wall of the egg case, and even at this stage the rudimentary basal disc and flanges can be separately discerned in it. When the capsule is pushed through the lower half of the gland, collecting the material for its base as it goes, a second period of secretion is initiated and a layer of almost pure protein secretion is applied over the already formed walls and also over the top of the plug embedded in them.

Discussion

In the majority of the British Stenoglossa the development of which has been studied, there is complete suppression of the free living larval stage, the young emerging from the capsule as a miniature of the adult. In some of them, however, such as *Nassarius*, the capsule contains up to a hundred eggs and these develop into planktonic larvae. In *Ocenebra* about a fifth of this number of eggs is present in one capsule and there is sufficient nourishment for all to develop as far as the crawling stage. The newly laid egg capsules of *Nucella* and of *Buccinum undatum* contain several hundreds of eggs, but the supply of nourishment within any one capsule is insufficient for all the eggs to complete development, and, as a result, the most precociously developed embryos feed on the less advanced. In *Littorina* (Linke, 1933) and the majority of other prosobranchs each egg is surrounded by its own supply of nutrient albumen with which it is encased in a protective shell: it is the loss of this individual shell—uniting all the eggs in a common chamber—which has permitted the evolution of embryonic cannibalism in the Stenoglossa and has, among other factors, contributed to the suppression of a free larval stage.

The egg capsules of the Stenoglossa which have been investigated are very similar in structure. The variations in shape are due not to any fundamental differences in the structure of the genital ducts themselves, but to differences in the shape of the ventral pedal gland which moulds the capsules passed to it in an unfinished condition from the capsule gland. The wall of the capsule is highly elastic and has a fibrillar appearance due to the mixing together of the various secretions from the capsule gland. One end is anchored to the substratum and at the opposite end is a circular aperture in the wall which is filled by a mucous or mucoid plug. Ankel (1937) has shown that in *Nucella* this plug is dissolved by the young animals when they are ready to hatch, and it is probable that in the other species the animals have a similar means of escape, for in empty, discarded, capsules the wall is always intact and the plug gone.

The ingesting and resorptive gland of the female genital system is one of the more interesting features which call for discussion. The phenomenon of sperm and yolk ingestion does not appear to have been previously described in the Mollusca, although the ingestion of sperm has been demonstrated in other invertebrate phyla. In the bed bug, *Cimex*, Abraham (1934) has shown that the majority of sperms in the female are normally absorbed in the "Resorptionsorgane", previously known as the spermatheca (Cragg, 1923). Here they become motionless and disappear. Sperms are also taken up by leucocytes as they traverse the haemocoel on their way from the body wall to the genital tract. In *Peripatopsis* (Manton, 1938) there is no special sperm ingesting organ, but absorption takes place in the ovary and the sperm provides the ova with some of the nutriment necessary for growth. In both these instances there appears to be some special significance attached to the process, for it has been suggested that the normal development of the ova is dependent on sperm absorption (Cragg, 1923; Manton, 1938). Copulation is of frequent occurrence in both animals. An ingesting gland is well developed in *Nucella lapillus*, an animal in which copulation and capsule formation continue throughout the year; examination shows that an excess of sperm is often present in the female, whilst the frequency with which copulating pairs can be collected suggests that copulation is frequent in this species too. The excess sperms are absorbed as previously described, but whether the development of eggs to maturity is in any way dependent upon the amount of sperm absorbed, or whether absorption is merely to empty the ducts of unwanted sperm, is as yet unknown. In *Nassarius reticulatus* the gland is smaller and probably of less importance in the functioning of the reproductive system. Specimens of *Buccinum* which were collected and sectioned during April show that the number of sperms in the gland is small as compared with the quantity of yolk present. During the breeding season the sperms in the genital duct of a female *Buccinum* are comparatively fewer than in *Nucella* and consequently the need for sperm ingestion may be less. The gland, however, is not reduced in size, since a second task—that of resorbing unused yolk granules—has been imposed upon it. This yolk must originate from eggs which for some reason are not included in a capsule. In *Ocenebra* during the breeding season yolk has also been found in the lumen of the gland and in the resorptive cells, but not in such quantities as in *Buccinum*. Yolk resorption may occur in *Nucella* and *Nassarius*, but has never been observed.

In the male system there is also a means of resorbing sperm. In the vesicula seminalis the epithelial cells frequently contain sperm in various stages of digestion. Linke (1933) observed this in *Littorina* and stated that the process continued during the whole period of sexual activity. This suggests that the production of sperm by the testis may be in excess of requirements, or that too great a number of sperms in the seminal vesicle may in some way upset the functioning of the system. The cells may have the ability of resorbing only senile sperm (though the presence of senile sperm so close to the testis seems improbable) and thus by a selective mechanism maintain within the duct an effective stock, or the absorption may be haphazard with the intention of safeguarding the duct against blockage. In neither male nor female is the ingestion of sperm restricted to that of apyrene spermatozoa: in both sexes, where two kinds do occur, each type is ingested.

The opening of the prostate into the mantle cavity is another character of the male genital system which needs some comment. In *Littorina* the male duct, anterior to the opening of the vas deferens into the prostate, is open throughout its length. The lower Stenoglossa, *Nucella* and *Ocenebra*, show evidence of the method by which this duct was closed during the course of evolution, but in them the closure is incomplete at one point, and a slit-like communication with the posterior end of the mantle cavity remains—in *Ocenebra* this is of quite considerable size. The cilia which direct the sperm through the prostate beat away from this opening and during the actual transference of sperm to the female there is no leakage into the mantle cavity. In *Nassarius* and *Buccinum*, in which the prostate is in the form of a narrow and highly muscular duct, there is an opening in a corresponding position, but it lies at the end of a short duct which leads from the prostate to the mantle cavity. In *Buccinum* the opening of the vas deferens into the prostate is well in front of this duct, so that normally the sperm, which is rapidly passed forward by peristalsis, is prevented from escaping. Although there is an evolutionary trend towards the complete closure of the male duct the persistence of the opening in these higher forms at once suggests that it plays some important role. It is conceivable that it functions as a safety valve, for if during the act of copulation the male is disturbed and forced to withdraw within its shell, sperm distending the prostate would be enabled to escape into the mantle cavity, so reducing the pressure in the gland and facilitating a more speedy withdrawal to safety. There is no evidence that excess sperm liberated from the vas deferens is at any time absorbed by the cells lining the more anterior regions of the male duct; it may be that it is always released to the mantle cavity. On one occasion when the shell of a male *Buccinum* was removed by bone forceps sperm was actually seen to escape in this way.

In the female the gonopericardial duct opens at the junction of the oviduct with the albumen gland. It is found in a comparable position in female *Littorina*. In the Stenoglossa and *Littorina* it is absent in the male, as also in *Calyptraea sinensis* and *Crepidula unguiformis* (Giese, 1915). In the mature male of *Ocenebra erinacea* there is evidence of its previous existence in the presence of a short diverticulum given off from the vas deferens and connected to a prominence on the pericardial wall by a band of dense connective tissue and muscle fibres. In *Littorina* there is no diverticulum, but a similar strip of connective tissue is developed. Linke (1933) suggests that the persistence of this duct in the female is connected with the fact that the eggs are too large to pass through it, and that it has been abolished in the male to prevent sperm escaping. This may explain its distribution in the Stenoglossa, but in *Ocenebra* sections of the duct show that its dimensions when the muscles are relaxed would allow the passage of eggs, and in one specimen of *Buccinum*, which admittedly was under abnormal conditions (affected by the strain of having the shell removed), eggs were seen to pass from the oviduct to the pericardial cavity.

The gross structure of the male genital systems of the stenoglossan prosobranchs described here agrees in yet other respects with that of the male systems of the above-mentioned mesogastropods. In *Calyptraea*, *Crepidula* and *Capulus* (Giese, 1915), and in the three species of *Littorina* described by Linke (1933), the upper part of the vas deferens functions as a seminal vesicle, and from this, during copulation, sperm is liberated to the true vas deferens, a short ciliated duct. In all of these molluscs, however, the seminal duct anterior to the vas deferens is represented by an open groove. A bilobed prostate is developed in *Littorina* and this is homologous with the prostate of the Stenoglossa.

So far as the female is concerned the genital systems of the Mesogastropoda and the Stenoglossa conform to the same fundamental plan—a narrow proximal oviduct opening into a wide glandular distal section with a spermatheca lying near the point of junction. It may be pointed out that the male tract can be split into the same two divisions and, with the exception of the males of *Nassarius* and *Buccinum*, the thickening of the walls of the glandular parts in both sexes is restricted to the lateral walls, leaving narrow dorsal and ventral areas where few glands occur. In animals with an open genital groove the dorsal area appears as a groove separating the right and left glandular lobes, along the free edges of which runs on each side a thinner marginal strip, representing the ventral areas. When fusion of the lips of the groove has converted it into a closed tube the former persists as the narrow dorsal wall of either the prostate or capsule gland, and the two latter join one another to form the ventral channel. In the mesogastropods the receptaculum seminis is situated at the posterior end of the glandular uterus; in *Littorina* it is in the form of a small pouch embedded in the albumen gland; in *Calyptraea* and *Crepidula* it is represented by a number of blind tubules. No mention is made of any sperm-ingesting epithelium in these snails, so that there would appear to be no method of utilizing the waste genital products. The ingesting gland of the Stenoglossa occupies a comparable position and is homologous with the receptaculum—the duct of the gland still functions as an area for the storage of spermatozoa, but at its distal end a large number of glandular tubules are developed and in the walls of these sperm and also yolk granules may be digested.

The various types of gland cells of the thick-walled uterus are in each genus mapped out according to a definite plan. In *Calyptraea* the cells are of three types: some pour a fibrous secretion over the eggs, which may be similar to the conchiolin from the capsule gland of the Stenoglossa, and others are mucous cells. Giese was unable to trace the various types of secretion in the fully formed capsules. In *Littorina* the albumen gland occupies a posterior position. It is homologous with that of the Stenoglossa, and fertilization occurs in both within the lumen of the gland. The fertilized eggs of *Littorina*, each coated with albumen, pass next to the shell gland, a structure which is not developed in the Stenoglossa, for in them the individual eggs are not enclosed in a shell, but lie freely in a common mass of albuminous secretion. In *L. littorea* and *L. obtusata* the jelly gland, which lies anterior to the shell gland, is homologous with the capsule gland of the Stenoglossa. In *L. rudis* the jelly gland has been modified to form a brood pouch in which the embryos develop into miniature adults. Whereas in the Stenoglossa the wall of the capsule is secreted around the egg mass in the capsule gland, Linke states that in *L. littorea* the jelly gland secretes a gelatinous fluid around the eggs, which have already been enclosed in a shell, and that the outer wall of the egg case is formed in and moulded by the ovipositor, which is situated on the right side of the head. The wall is tough and shows spiral markings. Sections of the jelly gland of this species show that it is made up of cells producing a protein secretion and of some mucous cells—hence in histological structure it resembles the capsule gland. In the Stenoglossa there is no definite structure to which the name ovipositor may be applied, but the same region at the right side of the head passes the capsule to the ventral pedal gland and so may be regarded to some extent as its homologue. The ventral pedal gland takes over the task of moulding the capsule. A bursa copulatrix lies at the anterior end of the jelly gland in *Littorina* and sperms are conducted from this pouch to the receptaculum by way of two longitudinal folds: these are ciliated and the sperm travels beneath them in the direction of the ciliary currents. In the Stenoglossa similar longitudinal folds separate a ventral channel from the lumen of the capsule gland, and along this the sperms are passed by peristalsis, assisted by their own activity, to the duct of the ingesting organ. No bursa is developed in *Calyptraea*, *Capulus* or *Crepidula*, for in each of them the penis deposits the sperm directly into the receptaculum.

So far as the interrelationships of the various gastropods dealt with in this paper are concerned, the conclusions which can be drawn from the comparative structure and function of the reproductive system are the same as have been arrived at by a study of other systems—that *Nucella* and *Ocenebra* belong to a more primitive group than *Buccinum* and *Nassarius*. This is seen particularly clearly in the persistence of indications of an open male duct in the two former genera, and, in *Ocenebra*, in the presence of a well-formed vestige of a gonopericardial duct in the male. In other respects the two groups appear to have attained a very close degree of similarity and this extends even to the habit of embryonic cannibalism. Since some members of each group have this habit and others have not this must be an example of parallel evolution.

Nassarius appears to have retained a primitive feature in still possessing a free-swimming veliger, although in other respects, such as the ventral pedal gland, it appears to be less primitive than *Buccinum*, but the gland in the latter may owe its primitive structure to degeneration. The other members of the group have sacrificed the benefit of wider distribution conferred by free larvae for the greater certainty of the young developing to a crawling stage and of reaching the correct habitat for adult life; this is ensured, first by the provision of eggs as food for the precociously developing embryos, and secondly by the fixation of the capsules on the shore in a suitable position for the young snails to start independent life.

So far as the remaining groups of the Stenoglossa, with the exception of the Toxoglossa, are concerned, little in the way of comparison can be made. The Volutacea include a large number of mollusca of the anatomy of which very little is known. The work of Küttler (1913) on *Oliva* indicates that there is a close similarity between this animal and the Buccinacea. In the Volutids, on the other hand, the brief note by Woodward (1901) would suggest that the conditions of the male genital tract are rather more primitive than occur elsewhere in the Stenoglossa, since he describes an open seminal groove running across the mantle cavity and along the penis. The description is too short to allow one to decide whether the condition resembles that found in *Calyptraea* or that in *Nucella*.

Taking all into consideration, however, the fact remains that the Stenoglossa are a highly specialized group of prosobranchs. This can be seen not only in the highly modified genital system, in the specialization of the egg case and embryonic mode of life, but also in the structure of the nervous system (Bouvier, 1887), in the elongation of the proboscis (Amaudrut, 1898) and in the complexity of the oesophagus (Graham, 1941).

SUMMARY

The foregoing pages include an account of the anatomy and histology of the male and female genital ducts of *Ocenebra erinacea*, *Nucella lapillus*, *Nassarius reticulatus* and *Buccinum undatum*. Observations have also been made on the mode of functioning of the ducts and the formation of the egg capsules.

In both male and female it is possible to divide the genital ducts into a narrow and thin-walled proximal section which leads from the gonad and opens into a thick-walled glandular distal region, and in this respect the Stenoglossa agree with the mesogastropods. Near the junction of these two portions, in the female, arises the gonopericardial duct, which puts the genital duct into communication with the pericardial cavity; in males this structure is lost or reduced to a vestige lying in a similar position.

In males the upper part of the proximal region of the genital duct is used as a vesicula seminalis and the epithelium is capable of taking up and digesting effete or superfluous sperm. Sperms are passed from it into the anterior, distal region during the act of copulation, and secretion from the glands is mixed with them and provides the medium in which they are transferred to the female. In *Ocenebra* and *Nucella* this glandular region, the prostate, extends only as far as the opening of the mantle cavity, from which a continuation of the duct, without glandular walls, runs to the penis. The entire distal half of the male duct shows evidence of having been derived from an open seminal groove such as is found in *Littorina*. The transformation is incomplete at the posterior end, where the prostate opens directly to the mantle cavity. In *Nassarius* and *Buccinum* the prostate extends to the base of the penis and there is a similar communication in the form of a narrow duct. In all the species, seminal fluid may, if necessary, be lost through this opening.

In females the whole of the proximal part of the oviduct serves to carry eggs from the ovary to the glandular distal section, a sphincter preventing their entry into the gonopericardial duct. The distal region may be divided into (i) the albumen gland; (ii) the capsule gland; (iii) the vestibule; (iv) the vagina; (v) between the albumen and capsule glands, the spermatheca, and (vi) the bursa copulatrix.

The albumen gland produces an albuminous fluid by which the eggs are surrounded, and fertilization occurs in this region. The capsule gland secretes around the mass of eggs and albumen the walls of the egg case composed of mixed protein and mucoid materials, the process of capsule formation being described in detail. The plug, of mucus, is produced by localized regions of the gland, the posterior mucous tips, and is moulded into position in the capsule by special muscular strips. The bursa copulatrix receives the seminal fluid from the male in copulation. The sperm becomes attached to the walls and, in *Buccinum* at least, the prostatic secretion is absorbed by amoebocytes. Later the sperms migrate, partly by their own activity, partly by muscular or ciliary action on the part of the female, along the ventral channel of the capsule gland to the spermatheca. They are stored here by being attached to the wall until required, and some storage may also occur in the upper end of the ventral channel. The spermatheca is provided with several tubular outgrowths distally, and in these ingestion and destruction of superfluous spermatozoa occur. In *Ocenebra* and *Buccinum*, and probably in the two others as well, a resorption of stray particles of yolk occurs in the same epithelium. No connexion between this and the maturation of the eggs has been noticed, nor is the ingestion limited to apyrene spermatozoa. No shell gland is present and the eggs are therefore enclosed in a common mass of albumen, a fact which permits of embryonic cannibalism.

The egg capsules, roughly formed by the glands at the base of the oviduct, are passed along a temporary groove on the right side of the foot, perhaps homologous with the ovipositor of mesogastropods, to the ventral pedal gland in the middle of the sole of the foot. Here they are manipulated into their definitive shape, their walls are hardened and they are cemented to the substratum. The gland occurs in females only. In both sexes in *Ocenebra* and *Nucella* a second ventral pedal gland of obscure function lies directly in front of it.

The relationships of the Stenoglossa amongst themselves and with the lower gastropods are discussed.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Resistance of Nerve in Relation to Interpolar Length

By J. F. Danielli

Journ. Physiol., Vol. XCVI, 1939, *pp.* 65–73

Assuming that a nerve may be regarded as a conducting core surrounded by a resistant sheath, lying in a conducting medium, conductivity experiments have been made following Rushton's treatment, by which the several resistances can be determined. Errors, probably caused by uneven distribution of water over the surface of the nerve and by uneven contact with the electrodes, are so large that a rigorous test cannot be obtained for this hypothesis. The results obtained, however, are compatible with this theory.

Very large errors also enter into the measurement of *R*, the resistance across a nerve sheath. It has, however, been possible to show that for frog nerve the maximum change in *R* for a *p*H change from 6 to 8 is less than ± 10% of the value at *p*H 7. Addition of Ca or K to bring the total in Ringer to ten times normal does not change by more than ± 30% of the value found in normal Ringer. Both Ca and K in these concentrations have very marked effects on the excitability of the nerve, so that it seems probable that the membrane on which these ions act gives rise to a relatively insignificant part of the total sheath resistance. J. F. D.

Factors in Cation Permeability

By H. Davson and J. F. Danielli

Biochem. Journ., Vol. XXXII, 1938, *pp.* 991–1001

The possibility that the high concentration gradient of K^+ maintained by the erythrocytes of many species against their surrounding plasma is due to an active metabolic process has been investigated. Metabolic poisons, such as CN, CO, urethane, and accelerators such as methylene blue and pyocyanine had no influence on the K^+ content of the non-nucleated erythrocytes of the rabbit and the nucleated cells of the goose and dogfish. Fluoride caused a small loss of K^+, but it was concluded that this is not associated directly with the inhibition of glycolysis produced by this substance.

The extent to which losses of K^+ may be caused by centrifuging the cells from serum or saline was studied. Direct proof of such losses was obtained with the erythrocytes of the dogfish and ox. The possible bearing of these effects on earlier work is discussed.

The question as to whether haemolysis, however produced, is preceded by a state of cation permeability was studied. Subhaemolytic concentrations of amyl alcohol, the dihydric phenols and guaiacol caused losses of K^+ from the rabbit erythrocyte; digitonin, Na cholate and oleate caused losses only when in haemolytic concentrations (these losses were over and above those due to the haemolysis itself); saponin produced no loss of K^+ in all the concentrations studied. Acidity and alkalinity were only effective under haemolytic conditions, whilst raising the temperature caused considerable losses of K^+; the penetration of K^+ into the erythrocytes of the ox from strongly hypotonic aqueous KCl was demonstrated. The haemolytic action of silver is preceded by an induced permeability to cations. J. F. D.

THE COLOUR CHANGES AND COLOUR PATTERNS OF *SEPIA OFFICINALIS* L.

By William Holmes

Proc. Zool. Soc. London, A, Vol. cx, 1940, *pp.* 17–36

The cuttlefish, *Sepia officinalis*, has the power to change its colour more strikingly developed than any other animal known. As the result of the variety of its chromatophore pigments, and the neuro-muscular nature of the chromatophore control, it can show a number not only of colours but also of patterns of colour on the surface of its mantle and head; and each pattern appears under specific conditions of stimulation. And its colour behaviour is made still more impressive by the fact that one pattern can be changed for another entirely different in times of less than a second.

The weight of evidence is in favour of the view that its powers of colour change and pattern production endow *Sepia* with a system of protective coloration unique in its complexity. Protection is afforded chiefly by various concealing patterns, each produced in the appropriate environment. But there are also patterns which may well be directed towards "terrifying" a predator. Further, it is suggested that in its power to change conspicuous patterns rapidly *Sepia* is provided with a protective colour device which does not fall into any category of Poulton's classification. This device depends on the fact that the pattern change gives the impression of rapid movement, and this movement will probably cause a "flight reaction" in the predator. For it is commonly observed, in fish for example, that any sudden movement in the visual field causes the animal to withdraw. W. H.

The Myenteric Nerve-plexus in some Lower Chordates

By **P. Kirtisinghe**

Quart. Journ. Micr. Sci., Vol. LXXXI, 1940, *pp.* 521–39

Preparations of the myenteric plexus in a few teleosts, in *Scylliorhinus canicula*, and in *Amphioxus*, stained chiefly with methylene blue and silver nitrate methods, showed a much simpler arrangement than in the higher forms. The neurons of the plexus in teleosts belong to Dogiel's types I and II, in *Scylliorhinus* only those of type II were found. In *Amphioxus* bipolar nerve cells are definitely present in addition to the stellate ganglion cells described by Boeke. Synapses between (*a*) the pre-ganglionic fibres and the enteric neurons, and (*b*) the enteric neurons themselves are figured and described. No connexion between the system of "interstitial cells" of Cajal and the enteric nerve-plexuses was observed. The enteric plexuses of fish and *Amphioxus* are not in the form of a nerve-net. It is not possible to compare the enteric plexus of *Amphioxus* with the system of "interstitial cells" of the higher chordates. The stellate ganglion cells of *Amphioxus* are comparable to the neurons of type I in the higher forms. P. K.

Reproduction of the Dogfish

By **H. Metten**

Phil. Trans. Roy. Soc. London, B, Vol. CCXXX, 1939, *pp.* 217–38

Migration of ova from the ovary to the coelomic opening of the oviducts in *Scylliorhinus canicula* is entirely dependent upon ciliation, as it is in Amphibia. The entire peritoneal wall and many abdominal viscera of an adult female are ciliated. The cilia, which are absent in the male and immature female, direct a current of coelomic fluid towards the ostium. An ovum excised from the ovary of one adult female and inserted through an abdominal incision into the peritoneal coelom of another will undergo migration towards the ostium. The speed of the migration depends upon the region into which the ovum is introduced. An ovum placed in the bridge joining the two liver lobes will traverse the hepatic sinus, enter the ostium and descend an oviduct as far as the top of the oviducal gland, in three hours. There are no muscular movements of any of the organs concerned.

In addition to its function of secreting albumen and the egg-case, the oviducal gland of the dogfish is a receptaculum seminis. By triturating a portion of the shell-secreting zone of the gland in saline solution, active spermatozoa can be seen at all times of the year. There does not seem to be any sharply defined breeding season, but an increase in breeding activity is noticed in the spring. The oviducal glands of all adult females contain spermatozoa, but the sperm content is very variable. In preparations of resting glands, spermatozoa can be seen in the shell-secreting tubules, whilst in actively secreting glands, a proportion of these spermatozoa are emitted in the shell material. In this way fertilization of an ovum is practically ensured. H. M.

ALGAL PRODUCTION AND THE FOOD REQUIREMENTS OF A LIMPET

By H. B. Moore

Proc. Malacol. Soc., Vol. XXIII, 1938, *pp.* 117–18

On the outer wall of the new swimming pool at Tinside, Plymouth, a thick algal felt had developed, and in this a number of limpets (*Patella vulgata*) had eaten clear patches. By measuring the sizes of the cleared areas and of the limpets on them it was estimated that in their first year these individuals require an area of about 75 sq. cm. per c.c. of limpet to support themselves, food being provided both by clearing the edges and by cropping the already cleared area. H. B. M.

THE COLONIZATION OF A NEW ROCKY SHORE AT PLYMOUTH

By H. B. Moore

Journ. Anim. Ecol., Vol. VIII, 1939, *pp.* 29–38

A newly made stony beach and a concrete wall at Tinside, Plymouth, were kept under observation for two years after their completion. The time of arrival, abundance and growth-rate of the colonizing species of animals and algae were noted and compared with a neighbouring long-established beach. The results indicated rapid colonization by a few hardy species and very slow colonization by most others. It appeared further that the latter were deterred, not by over-crowding, but by some environmental condition particularly unfavourable to the young stages, since those individuals which succeeded in establishing themselves showed a quite normal growth rate. H. B. M.

FURTHER OBSERVATIONS ON THE COLONIZATION OF A NEW ROCKY SHORE AT PLYMOUTH

By H. B. Moore and N. G. Sproston

Journ. Anim. Ecol., Vol. IX, 1940, *pp.* 319–27

The state of colonization of the flora and fauna on a new concrete wall and stony beach at Plymouth after six years is described, for comparison with a previous survey made during the first two years (Moore, 1939: see above). Fourteen species of Algae and seventy-one species of animals were found. The highest and lowest zones of the beach appear to have developed more quickly towards a climax than the intermediate zone, in which environmental conditions are less favourable than in the others. N. G. S.

SOME OBSERVATIONS ON THE GROWTH OF *PERINGIA ULVAE* (PENNANT) 1777 IN THE LABORATORY

By Anne Rothschild and Miriam Rothschild

Novitates Zool., Vol. XLI, 1939, *pp.* 240–7

A comparison was made between the growth-rate of *Peringia ulvae*, a brackish water Gastropod, collected from two different habitats and kept in the laboratory in (*a*) glass bowls, and (*b*) glass tubes. It was found that the snails kept in the bowls grew considerably faster, and that their size, consequently, is no criterion of their age. It was also found that apart from the different rapidity of increase in size the specimens in bowls and tubes displayed certain differences in their growth curve. When reared in the laboratory specimens collected from the mud-flats and saltings, which in nature differ from each other in size, shell shape, shell colour and shell texture, show no such variation, and it therefore seems probable that these differences are due to environmental rather than genetic factors. The snails parasitized by trematodes grew faster than uninfected specimens, thus supporting the theory that these infections cause increased growth. It was also found that uninfected females grew faster than uninfected males, and it seems probable that the larger size of females in nature is due to increased growth-rate rather than a longer life. M. R.

A NOTE ON THE LIFE CYCLE OF *CRYPTOCOTYLE LINGUA* (CREPLIN) 1825 (TREMATODA)

By Miriam Rothschild

Novitates Zool., Vol. XLI, 1939, *pp.* 178–80

The life cycle of *Cryptocotyle lingua* was demonstrated experimentally in the laboratory. The cercariae were found parasitizing *Littorina littorea*; a number of fish, more especially in-shore forms such as wrasse, rockling, blennies and gobies, served as the intermediate host. The cercariae encysted in the fins and skin, and in those fish which are probably the natural hosts, stimulated the formation of pigment deposits round the cysts. The infected fish were kept in the laboratory a fortnight, and then fed to laboratory-reared albino rats, ducks and black-headed gulls. The adult worms were recovered from the intestine of the latter birds a month later. It was found that the behaviour of the cercaria is of importance for distinguishing between closely related species. The fertility of single infections proved to be very high. *Littorina* in the laboratory emitted cercariae for periods extending over three years, averaging about 300 cercariae per day. M. R.

ELECTROLYTE CONTENT AND ACTION POTENTIAL OF THE GIANT NERVE FIBRES OF *LOLIGO*

By D. A. Webb and J. Z. Young

Journ. Physiol., Vol. XCVIII, 1940, *pp.* 299–313

Previous attempts to discover the physico-chemical basis of the resting and action potentials of nerve fibres have been handicapped by the presence in ordinary nerve trunks of connective tissue and intercellular fluid, which reduce the potentials by short-circuiting and introduce into the analyses an error of considerable but unknown magnitude. With the giant fibres of *Loligo* it is possible to isolate a single fibre, measure its resting and action potentials, and then submit it to chemical analysis. It is shown that the observed action potentials approximate closely to the diffusion potentials which would be produced if the bounding membrane of the axoplasm were permeable only to potassium ions, except during the passage of the impulse, when it is presumed to be freely permeable to all ions. The potentials may therefore be attributed largely, though not entirely, to the high concentration of intracellular potassium (0·28 *M*, as against 0·01 *M* in sea water) and the differential permeability of the axon membrane. Chloride is present in the axoplasm at a concentration of 0·11 *M*, which, though only a fifth of the concentration in sea water, is far higher than the values reported for the cells of vertebrates. The electrolyte balance-sheet of the axoplasm reveals large osmotic and anion deficits, indicating the presence in considerable quantity of anions (and perhaps non-electrolytes also) whose nature is not yet known. D. A. W.

OBSERVATIONS ON THE COELENTERATE NERVOUS SYSTEM

By the late H. H. Woollard and J. A. Harpman

Journ. Anatomy, Vol. LXXIII, 1939, *pp.* 361–2

DISCONTINUITY IN THE NERVOUS SYSTEM OF COELENTERATES

By the late H. H. Woollard and J. A. Harpman

Journ. Anatomy, Vol. LXXIII, 1939, *pp.* 559–62

The histology of the nervous system in *Cyanea lamarcki* and *Chrysaora isosceles*, and in the tentacles of *Actinia equina* and *Tealia felina*, has been studied by the methods of methylene blue and gold chloride. In the jelly-fishes, nerve cells and fibres are most abundant in the sub-umbrellar region close to the sense organs.

The nerve cells are usually bipolar; multipolar cells are infrequent. The cells contain granules (stainable with methylene blue) which extend for a short distance into the cell processes. There is no differentiation into dendrites and axon, and no neurilemma is present.

The fibres usually appear varicose. The larger varicosities, at least, are due to toxic effects of the methylene blue. The fibres may be thick or thin, the distinction depending on the distance of the fibre from the parent cell. At the cell all the fibres have the same thickness.

The fibres may run parallel, cross each other obliquely or transversely, or commonly one fibre twists itself round another. Where the fibres cross each other they may lie in intimate contact, one of them showing a thickening. Fibres may also lie in close contact with the surface of nerve cells. Sometimes an ending resembling a "bouton" was seen. Intertwining between the fibres is a characteristic and frequently observed relation. Fusion does not occur, no true nerve net being formed. The intertwining and other intimate contacts between nerve fibres and fibres and cells are believed to be of synaptic significance.

Pericellular nerve endings on muscle occur; they are comparable to the endings on unstriped muscle cells in mammals. Neuro-sensory cells were also observed, but the relation of their processes to the general nerve plexus could not be determined.

The present observations confirm the histological work of Schäfer (1878) and Bozler (1927) and correlate favourably with the physiological findings of Pantin (1935). They are not in accordance with those of the majority of observers, whose descriptions have led to the general acceptance of the faulty notion that the coelenterate nervous system is a nerve net. J. A. H.

ON THE MANTLE CAVITY AND ITS CONTAINED ORGANS IN THE LORICATA (PLACOPHORA)

By C. M. Yonge

Quart. Journ. Micr. Sci., Vol. LXXXI, 1939, *pp.* 367–90

Study of the course of the water currents in the mantle cavity of three species of the Chitonida and one species of the Lepidopleurida revealed that inhalant openings are created anteriorly or laterally by local raising of the girdle. The single exhalant opening is always posterior and confined to the region between the last pair of gills. The exhalant current carries with it the genital and excretory products and, in the Chitonida, the faeces. The functional division of the mantle cavity into inhalant and exhalant chambers is brought about by the bridging of the pallial grooves in the region of the pallial folds by the post-anal (and adanal) gills. Though modified the gills possess the typical structure and ciliation of true ctenidia. They are to be regarded as multiplied ctenidia and not as secondary structures. Four possible tracts of mucous glands in the pallial grooves are concerned with the consolidation of sediment; of these the pallial tracts are possibly homologous with the hypobranchial glands in the Prosobranchia. Osphradia, possibly homologous with those in the Gastropoda, occur in the majority of the Chitonida; in the Lepidopleurida they are replaced by branchial and lateral sense organs. All are similar in structure and as likely to be concerned with the estimation of sediment as with olfactory functions previously ascribed to them. The Loricata probably evolved between tide-marks, their characteristic structure being admirably fitted for life on the shore.

C. M. Y.

THE PROTOBRANCHIATE MOLLUSCA; A FUNCTIONAL INTERPRETATION OF THEIR STRUCTURE OF EVOLUTION

By C. M. Yonge

Phil. Trans. Roy. Soc. London, B, Vol. CCXXX, 1939, *pp.* 79–147

The Protobranchia represent the one unquestionably natural group within the Class Lamellibranchia. The large foot permits movement into and through a soft substratum. Feeding, except in the modified Solenomyidae, is by means of the palp proboscis which collects organic detritus. In these respects the structure of the Protobranchia probably corresponds closely to that of the primitive Lamellibranchia, the evolution of the palp proboscides permitting retreat of the mouth from the substratum and the enclosure of the body within the bivalve shell. This involved life on a soft substratum, so that the apparently specialized foot is probably primitive for the Lamellibranchia.

The Nuculidae are the most primitive of existing Protobranchia, having an anterior inhalant current, primitive ctenidia essentially respiratory in function, and hypobranchial glands. Conditions are essentially similar in the Solenomyidae, but the ctenidia are enlarged as feeding organs. The Nuculanidae possess highly specialized "pumping" ctenidia forming delicate septal membranes, the filaments being united by complex ciliated junctions to each other and to the siphonal septum. The inhalant current is posterior and siphons occur which in different species show interesting stages in the conversion of ciliary into tissue junctions. Rejection of sediment from the mantle cavity is by cilia, except in the Solenomyidae, where it is brought about by the intucking of the ventral, uncalcified regions of the shell.

The alimentary canal is correlated in structure with the nature of the food particles collected by the palps. There is a dorsal crushing region lined with chitin and a ventral "style-sac" region which secretes mucus. Particles are embedded in this to assist trituition and the formation of faeces. There is no amylase and so no extracellular digestion. The digestive diverticula are organs of intracellular digestion. They consist of paired masses except in the Nuculanidae, where there is an additional mass of wide diverticula opening near the entrance of the oesophagus, and into which larger particles enter. There are no wandering phagocytic cells. The intestine and rectum are concerned exclusively with the consolidation of faeces.

The nature, interrelationships, systematic position and evolution of the Protobranchia are discussed. C. M. Y.

FUSED NEURONS AND SYNAPTIC CONTACTS IN THE GIANT NERVE FIBRES OF CEPHALOPODS

By J. Z. Young

Phil. Trans. Roy. Soc. London, B, Vol. CCXXIX, 1939, *pp.* 465–503

A full description is given of the system of giant nerve fibres by means of which *Loligo* produces its rapid movements through the water. The whole system is operated by a pair of very large cells which lie in a hitherto undescribed lobe, the lobus magnocellularis. This lobe contains other large cells controlling the movements of the arms, and thus constitutes a higher motor co-ordinating centre. A peculiar feature of the giant fibre system is that in two places the processes of separate nerve cells fuse completely, contrary to the doctrine of the neuron theory. But in other parts of the system there are synaptic junctions at which it is very clear that no fusion takes place. The neurons between which fusion does occur always work together in life, and the case is therefore the exception which proves the rule of the neuron theory, showing that nerve fibres can fuse, but normally do not do so unless they invariably work together. The whole giant fibre system shows the extreme development of a neuromuscular mechanism for producing movement rather than tonus. Contractions are initiated by the action of the minimum possible number of motor units, and a single impulse is able to set the whole system in action. Such a system, though not well suited for the production of finely graded movements, allows the development of few, large and hence rapidly conducting fibres. The combination of speed with fine gradation of movement is only possible in animals, such as Vertebrates, in which rapid conduction is obtained by thickness or special structure of the myelin sheath, rather than by increased diameter of the fibres. J. Z. Y.

ARTHUR JAMES SMITH

ARTHUR JAMES SMITH (1871–1941)

A Personal Tribute by E. J. Allen

Those who have known the Plymouth Marine Biological Laboratory at any period during the last forty-five years will have learned with regret that our Chief Laboratory Attendant, Arthur James Smith, died on 29 January, after a trying illness which had lasted for more than a year. He took up the post which he held so successfully for so long in April 1895, about six months after my own appointment as Director. Trained at the Cambridge Zoological Laboratory, largely under the special care of Adam Sedgwick who always took a close and friendly interest in him, he owed much to the help he received from Brockett, the head attendant at Cambridge, to whom so many men in Zoological and other University Departments throughout the country were in the same way indebted.

When Smith arrived at Plymouth he already knew the Laboratory, having spent some weeks there the year before collecting and preserving material for use in Cambridge, and it was then, when I was working on the nervous system of embryonic lobsters, that I first met him.

From the beginning his work at the Laboratory was most successful and as the years passed he undertook the oversight of more and more of the routine activities of the whole establishment. He gradually acquired an extensive knowledge of the marine fauna and flora of the district and he was always eager to increase it and bring it up to date. For this he had many opportunities, since specialists in the systematics of particular groups, both visitors to the Laboratory and members of the scientific staff, were only too glad to repay the trouble he took to keep them supplied with material for their work by discussing with him the recent advances in their subjects. He had a very keen eye for discriminating species and was an excellent collector, especially on the shore and when examining material brought in from the daily work of our fishermen.

A group in which he took special interest was the Coelenterata, and the Ray Society Monograph on Anemones by Stephenson owes a good deal to his keenness and help. He made it, too, a regular practice to keep in touch day by day with the local fish market and with the fishermen and other workers there.

Smith brought with him from Cambridge a high degree of technical skill in dissection combined with considerable knowledge of the methods of preservation of animal tissues for section cutting or for museum purposes. This was greatly developed as his experience grew at Plymouth in the department for the sale of specimens, which was in his charge. It was well shown also by the ability he displayed in preserving and mounting specimens for exhibitions. An exhibit prepared largely by him attracted much attention at

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the St Louis Exhibition in 1903. This was subsequently purchased and remained in the United States. Other exhibits arranged under his supervision were shown at the Yachting and Fisheries Exhibition at South Kensington in 1897 and the Oceanographical Exhibition at Marseilles in 1906. In the latter case he travelled with the exhibits to France and himself set them up there. He also took a great part in preparing and setting up the exhibits of marine animals which have been a popular feature for so many years at the Annual Soirees of the Royal Society at Burlington House. His work in all these directions was remarkable for his great care over details: nothing short of the best would ever satisfy him.

Amongst other assets was a good share of business ability, and for some years, in addition to his other duties, he had charge of the office, and himself kept the detailed accounts called for by the varied activities of the Marine Biological Association. His work in this direction always met with the full approval of the professional auditors.

Smith was essentially a man of action—a man who could do things. There was in him a fundamental honesty of mind and character which permeated his whole life, and it was this, added to a general desire to help the scientific work of the Laboratory, that made him of such great value in the post he held. He gave of his best to a long and varied succession of biologists from all parts of the world by whom he will always be remembered with gratitude, and this feeling will be shared in a very special way by those who have been fellow workers with him at the Laboratory.

Nothing I can say can express my own personal debt for his life's work and the help and kindness I have received from him. Always reliable, always willing to undertake new responsibilities, there were few things connected with the details of administration that could not safely be left in his hands.

ON CHANGES TAKING PLACE IN SEA WATER DURING STORAGE

By H. W. Harvey, Sc.D.

Hydrographer at the Plymouth Laboratory

(Text-fig. 1)

Whipple reported in 1901 that when tap water was filled into glass bottles, the number of bacteria fell during the first 3–6 hr. by 10–25 %, and later increased by many hundred per cent, with a reduction in number of species. This rise in bacterial numbers was several times greater in small than in large bottles, and was reduced or even nullified if the water was kept agitated. A similar rise in bacterial numbers takes place when sea water is stored in glass vessels. Waksman & Carey (1935) found that multiplication took place in Seitz and in colloid-filtered water which had been inoculated with raw water, and from the oxygen used calculated that the rapid growth of bacteria breaks down about one-third of the organic matter in solution. Zobell & Anderson (1936) and Lloyd (1937), found a much greater increase in numbers of bacteria when the water was stored in small than in larger bottles, or in bottles where the water-glass surface area had been increased by filling the bottle with glass beads or rods. By observing the numbers at close intervals of time, Miss Lloyd found that the increase followed the course of a population curve; the peaks were determined by the volume of the container, but were not affected by the surface area of the water exposed to the air. Zobell & Anderson found that the peaks, or maximum number of bacteria found in the water, showed a rough direct proportion to the volume/surface area of the bottles, the maxima in small vessels being about twice the maxima in vessels ten times as large. They also observed that when the surface area was increased by a shallow layer of glass beads or silica grains, the resulting increase in numbers of bacteria was less than that calculated from the volume/area ratio. They concluded that not only this ratio but also the proximity of the main body of water to the glass surface played a part. The greatest increases were found in water between sand grains where populations of some twelve million bacteria per c.c. developed in water which maintained no more than a few hundred bacteria per c.c. in the sea.

Whipple had found that the marked difference between maximum bacterial population, which arose when tap water was stored in small and in large bottles, was much reduced if a small quantity of peptone (5 mg./l.) was added to the water. Zobell & Anderson noted that if nitrite and 10 mg./l. of peptone were added to sea water, there was a greater and more rapid loss of nitrite in smaller than in larger vessels, but no such difference when 100 mg./l. of peptone was added. It appears that the volume effect only occurs when bacteria develop in water containing food substances at very great dilution. This conclusion was also reached by Heukelekian & Heller (1940) who found no growth of the bacterium *Escherichia coli* in solutions containing 0·5 and 2·5 mg. of glucose and peptone, but growth did occur if glass beads were added. With 25 mg./l. growth did take place, and with concentrations greater than this the effect of adding glass beads faded out.

As Whipple had found for tap water, Zobell and Anderson found a reduction in number of species when sea water was stored. Some twenty-five to thirty-five species were generally found immediately after the water had been collected, falling to nine or ten species by the time bacterial numbers had reached a maximum and to no more than four or five species after the maximum population had declined. After this decline the population remained relatively high for a long period, the numbers fluctuating from a few thousand to over a hundred thousand per c.c.—a sample of sea water which had been stored at 2–6° C. for 4 years was found to contain 209,000 bacteria per c.c.

In Zobell and Anderson's investigation a series of experiments was made dealing with the effect of oxygen on the proliferation of bacteria in stored sea waters. No material effect was observed unless the water was less than 50 % saturated with air. Waksman & Carey, on the other hand, observed greater growth in fully aerated than in partially aerated water.

The oxygen content of sea water stored at 16° C. in glass-stoppered bottles of different capacities after 20 days, and the maximal bacterial population reached (after 3–5 days) in similar bottles. The water initially contained 3·46 c.c. O_2 per litre and 231 bacteria per c.c.

Volume of sea water (c.c.)	10	100	1000	10,000
O_2 per litre	2·59	2·90	3·68	4·17
Bacteria per c.c.	1,475,000	1,080,000	673,000	382,000

Zobell, 1936. *Proc. Soc. Exp. Biol.*, Vol. xxxv, p. 271.

Both Zobell & Anderson (1936) and Waksman & Renn (1936) observed that in full and stoppered bottles the consumption of oxygen continued undiminished for some time after the bacteria in the water had reached maximum numbers and while the population was falling. The former investigators have shown that great numbers of bacteria develop on the glass surfaces; one experiment indicated that within 24 hr. more than twice as many were attached to the surface of the glass as were in the water. This accounts for the continued consumption of oxygen after the number of bacteria in suspension have declined.

The proliferation of bacteria when water is enclosed in glass vessels and the effect of their size is attributed by Zobell & Anderson to the water-glass surfaces:

(i) Providing a resting place for periphytic bacteria, many marine species having periphytic tendencies and at least some being obligate periphytes. In this connexion it is pertinent that saprophytic bacteria are attached to suspended particles in the sea (Lloyd, 1930) and that bacteria are most numerous where plankton is most abundant, as observed by Waksman, Reuzer *et al.* (1933) who consider that 'bacteria exist only to a very limited extent in the free water of the sea, but are largely attached to the plankton organisms'.

(ii) Concentrating organic substances from very dilute solution on the surfaces owing to adsorption or other physical attraction.

(iii) Causing the diffusion of bacterial enzymes away from the cell, where it is attached to a solid surface, to be retarded; it has been generally observed that attachment to particles exerts a favourable influence on their enzymatic activity.

The second suggestion—that organic matter in solution is adsorbed on solid surfaces—is of particular interest. The authors state that the accumulation of a film of organic matter on glass slides soon after being submerged in sea water can be demonstrated by differential stains as well as by microchemical technique. Stark *et al.* (1938) also state that an accumulation of organic matter can be detected on glass slides which have been immersed for several hours in lake water. Their method of detection was based on the oxidation of a sulphuric acid-dichromate mixture. In neither of these communications is the exact technique described. The writer has been unable to obtain definite results on these lines, but a number of observations have been made which point to adsorption taking place.

It was noticed that when offshore water was kept in glass tubes a gelatinous ring of bacteria slowly developed at the meniscus, becoming apparent after five months (Harvey, 1925). This suggested that if their growth was due to local concentration of organic matter by adsorption, the latter took place to a greater extent at the meniscus, where potent physical forces come into operation, than on other parts of the glass surface. Experiments were therefore made to test this possibility.

If a clean glass or silica tube of about 1 mm. bore is dipped into sea water, the water rises in the tube and the meniscus takes up a position at a definite height above the surface, this height corresponding to a surface tension of approximately 72 dynes/cm. When the tube is either lowered or raised, the meniscus returns to this same height; it moves freely both up and down the tube, provided the glass is clean and moist. However, if a trace of various organic substances is dissolved in the water, such as 5 mg./l. of peptone or casein, it is otherwise. The water rises in the tube to approximately the same height as before, but if allowed to remain undisturbed for a few minutes and the tube is then lowered, the meniscus is lowered and is flattened owing to the lesser pull by the column of water below; finally the meniscus breaks free and rises again to the same height as before. If the water in the tube is covered with ether or benzene and allowed to stand, on lowering the tube the water-ether meniscus is lowered and becomes flatter, while the ether-air meniscus keeps its shape. It appears that a ring of organic matter is adsorbed on the glass where the meniscus has stood and this prevents water moving readily up the tube past the ring, but does not hinder the movement of water down the tube past the ring. Using more concentrated solutions, a series of rings were formed where the meniscus had stood in the tube for a few minutes, each ring acting as a valve allowing water to pass freely downwards but hindering its passage in the reverse direction. The contact angle of water advancing past the ring is considerable, while the angle when the water retreats remains at or near zero.

At somewhat higher concentrations, such as 50 mg. of sodium caseinate per litre, the solution rose in a clean tube to almost the same height as sea water without this addition, but when allowed to stand the column of liquid gradually fell. It appears that the water is repelled by the ring of adsorbed protein; a similar gradual fall took place when the column of liquid in the tube was covered with benzene.

The addition of 0·2 mg./l. of sodium caseinate, or less, could be detected by filling a short column into a clean capillary tube and allowing this to rest horizontally for a few minutes, after which a tilt of several degrees was required to start the column of liquid moving, whereas similar tubes with similar columns of filtered sea water required a tilt of *circa* one-twentieth of a degree. Using this method of detecting adsorbable substances in a liquid, the following experiment was made. A shrimp was ground with water, filtered, and the filtrate added to filtered sea water in the proportion of extract from 0·2 g. wet weight of shrimp per litre of water. A quantity of glass-wool, previously cleaned with concentrated sulphuric acid, washed and dried, was added to a part of this liquid. After standing for half an hour, a column about 1 cm. long was drawn into a clean glass capillary and moved to and fro in order to wet the glass surface. This capillary, and also one filled in the same way with a similar column of the liquid which had not been in contact with the glass-wool, were adjusted on a horizontal plate, so that the columns of liquid lay in the central parts of the tubes which were placed alongside each other. The plate was then slowly tilted and the angle of tilt at which each column started to move was noted. The plate was again levelled and a fresh pair of readings obtained:

Liquid ex glass-wool required	2° 36′	0° 39′	1° 18′	1° 3½′ tilt
Liquid not treated required	5° 12′	3° 24′	3° 24′	> 3½°

On repetition with a fresh pair of capillaries:

Liquid ex glass-wool required	0° 26′	1° 18′	0° 39′	1° 44′ tilt
Liquid not treated required	2° 10′	5° 12′	5° 12′	7°

Two hours later, that is, after the liquid had stood 2½ hr. in contact with the glass-wool, fresh tubes were prepared and gave results as follows:

Liquid ex glass-wool required	0° 13′	0° 13′	0° 26′	0° 26′	0° 13′ tilt
Liquid not treated required	0° 52′	2° 10′	2° 23′	0° 39′	0° 52′

This shows a marked falling off in the capability of the liquid which had stood in a glass vessel, and of that which had been in contact with glass-wool, to form a ring. A similar effect has been found in other experiments.

These experiments are interpreted as indicating that organic matter is adsorbed on a glass-water surface, the most rapid adsorption taking place where there is a glass-water-air or a glass-water-benzene or ether interface forming a meniscus. There is no indication of how much of the added organic substance is so adsorbed; it is not known to what particular substances the ring effect is due, but experiment suggests that it is not due to traces of oily impurities.

An attempt was made to evaluate the quantity of organic matter which was adsorbed from very dilute solution, such as 10 mg. of peptone per l., on a large surface exposed by glass-wool, about 50 sq. cm./c.c. of liquid. The most delicate method found for estimating the change, due to contact with glass-wool, was the quantity of dilute alkaline permanganate oxidized in 24 hr. at 30° C. by the liquid, the excess of permanganate being estimated by titrating the iodine set free on adding potassium iodide and a buffered mixture of sulphuric and boric acid. By acidifying with such a mixture, the trace of chlorine set free on acidifying permanganate in sea water was certainly reduced, if not eliminated; consistent triplicate titrations were obtained. The results of these experiments showed that only a small proportion of the oxidizable organic matter was adsorbed, possibly no more than the experimental error. The values obtained in the different experiments ranged from 0 to 7 % less in the liquid which had been in contact with glass-wool than in the control. Only a minute fraction of the added organic matter would be required to give a monomolecular layer; on the other hand, layers many molecules thick are known to build up on solid surfaces (Blodgett, 1935). Whether the presence of a monomolecular layer of nutrient on a surface would provide a sufficient local concentration of food to allow bacteria to grow, assuming it was rapidly renewed after being used, is not obvious.

It is an outstanding question why offshore sea water, which contains sufficient nutriment for the production of several million bacteria per c.c., and will in fact rapidly produce this population when in contact with clean sand grains, normally supports a population of no more than 10–200 bacteria per c.c. In addition to lack of solid surfaces, protozoa and other animals keep the bacterial fauna eaten down; this has been stressed by both Zobell and by Waksman & Hotchkiss (1937). The former investigator (1936) has also concluded that natural sea water contains a bacteriophage, or heat-labile substances inimicable to the growth of bacteria; added bacteria grew more rapidly in autoclaved than in Berkefeld filtered sea water.

Changes brought about by bacteria developing in stored sea water are the setting free of ammonia, phosphate and carbon dioxide, the interconversion of ammonia, nitrite and nitrate, and the utilization of oxygen.

The ammonia may either increase or decrease (Keys *et al.* 1935). When plankton is added to the water and it is stored in the dark, there is an increase in ammonia which later decreases as nitrite is formed; later nitrite decreases with an increase in nitrate (Von Brand *et al.* 1937). In a great number of cases no change has been found in the nitrate and nitrite content of water from the open sea during storage, even if enriched with ammonia, unless it was obtained close to the bottom or bottom deposit had been added to it (Harvey, 1926; Cooper, 1937). It appears that nitrite-forming and nitrite-oxidizing bacteria are not usually present in offshore water, free from plankton, unless collected close to the bottom.

The phosphate in solution increases slowly during storage, the increase being sometimes preceded by a decrease.

These changes are brought about by bacteria; however, there is evidence that both the setting free of phosphate and changes in the nitrogen cycle may take place to some extent without their aid. Kreps (1934) found that changes in ammonium and nitrate took place in inshore water which had passed a Seitz filter or been poisoned with mercuric chloride, and suggested that sea water, particularly water near the bottom where organic matter was decomposing, contains enzymes which cause these changes. Keys *et al.* have also noted changes in ammonium content of water which had been sterilized with mercuric chloride, while Newcombe & Brust (1940) have noted that saturating water with chloroform reduces but does not stop phosphate being set free during storage.

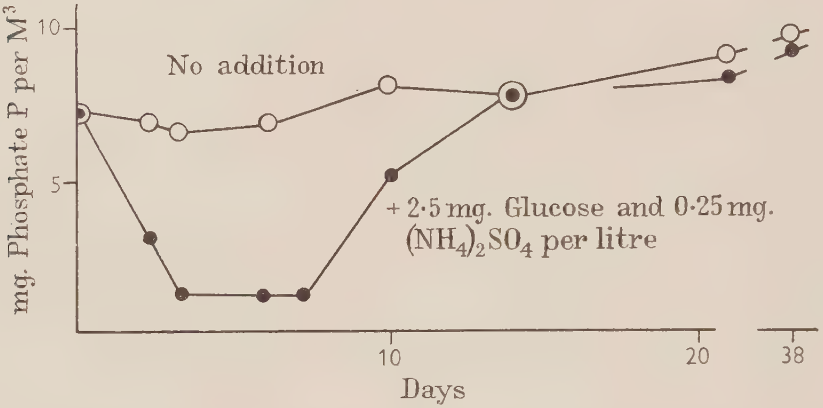

Fig. 1. Changes in phosphate concentration during storage in a sample of sea water, filtered through Whatman No. 3 paper. The lower curve shows the effect of increased bacterial growth due to the addition of phosphorus-free nutrient.

Several investigations concerning the bacterial decomposition of organic matter added to sea water throw light on changes taking place during storage. Waksman & Carey (1935) have come to the following conclusions. Bacterial growth which occurs in clear water from the open sea consumes a similar quantity of oxygen as that brought about by the addition of 2½ mg. of glucose per litre, and there are sufficient nitrogen compounds available for double the growth of bacteria which normally occurs when such water is stored. They found sufficient available phosphorus or nearly sufficient in the waters they used for the requirements of the bacteria; adding phosphate had no effect or only slight effect upon their growth. On the other hand, Keys *et al.* have noted instances where the addition of phosphate has increased the oxygen consumption of stored waters. Renn (1937) found that when glucose was added to sea water the resultant growth of bacteria assimilated significant quantities of phosphate, and that this was soon regenerated following their death. This accounts for the decrease in phosphate which sometimes precedes an increase when sea water is stored. The writer has made similar experiments, adding glucose and an ammonium salt in order to provide ample organic matter and nitrogen for bacterial growth, and in some experiments enriching the water with phosphate. The results showed rapid and complete, or almost complete, regeneration of the phosphorus which the bacteria had utilized—that is, dephosphorulation of their body substances during autolytic breakdown. Previous experiments had shown that bacteria in a sample of sea water rapidly set free phosphate from nucleic acid and from casein in solution, but not from glycerophosphate, suggesting that the phosphatase enzymes of the bacteria in these samples of water could only deal with certain types of organic compounds.

The breakdown of organic phosphorus compounds and setting free of orthophosphate is of particular interest. Russell (1935, 1936) has shown a close correlation between the maximum concentration of phosphate which has been found in the water of the English Channel off Plymouth during winter and the subsequent abundance of young fish. Indeed, the fluctuations in maximum phosphate and in abundance of animal life have now followed each other rather closely for a number of years in this area. Redfield *et al.* (1937) have succeeded in showing by chemical analysis that there is a considerable accumulation of organic phosphorus in solution in the water of the Gulf of Maine during the summer, nearly all breaking down to phosphate during the ensuing winter. However, in an experiment by the writer when summer sea water was filtered and stored, no such quantity of phosphate was set free during succeeding months as would be expected to take place if the water had remained in the sea with a bacterial population at least several hundred times less. Some observations by Cooper (1935) point in the same direction; two out of four samples of sea water which had only been freed from larger plankton organisms showed little change in phosphate content when stored for several months, yet, where animal plankton had been added to this water, not only was all the phosphorus added in this form regenerated as phosphate but a considerable quantity in excess. He concluded that this excess was set free from dissolved organic compounds present in the sea water. It seems reasonable to surmise that some species of bacteria grow on the relatively rich food provided by the added animal plankton, which do not proliferate when plankton-free sea water is stored, and that these are able to dephosphorulate a greater variety of organic compounds.

The observations which have been discussed suggest a problem which awaits, and would repay, solution. Russell's correlation has shown the value of a knowledge of the winter phosphate maximum of the water occupying an area, when studying the fluctuations in its total fauna from year to year; the same probably holds when studying differences between two bodies of water, due account being taken of such physical differences as the extent of vertical mixing. This winter maximum reflects the biological history and future of the water mass. The concentration of a conservative constituent such as chloride (salinity) reflects its physical past. To obtain the winter maximum requires the use of a ship over the period when the maximum is likely to occur, and exact colorimetric comparisons within a day or two of the samples being collected. The analysis of total organic and inorganic phosphorus, as made successfully by Redfield *et al.*, would require a team of workers to deal with the large number of samples necessary for a survey. Hence it would be particularly useful to reproduce in vitro the changes from organic phosphorus into ortho-phosphate which take place in the sea, presumably due, or mostly due, to the agency of bacteria, and which become apparent during autumn and winter when phosphate is not used as quickly as it is formed.

Summary

The rapid growth of bacteria when sea water is stored in glass vessels, and the possibility that this is brought about by the concentration of food by adsorption on the solid surface, are discussed. Some evidence is presented bearing upon such adsorption.

The regeneration of phosphate from dissolved organic phosphorus compounds in the sea and in stored water is considered.

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THE SOLUBILITY OF CALCIUM CARBONATE IN TROPICAL SEA WATER

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INTRODUCTION

In a previous paper (Smith, 1940*b*) the chemical changes observed in ocean sea water flowing across the shallow banks on the west coast of Andros Island (Bahamas) were reported. High salinities were produced by evaporation and calcium carbonate was precipitated. From the data obtained under natural conditions a maximum value of the solubility product constant of calcium carbonate in sea water was suggested. There was no reason to believe, however, that this value of the constant represented the true equilibrium conditions, and experiments have since been made in the laboratory with a view to bringing water from these banks into equilibrium with solid calcium carbonate.

Before describing this work it is necessary to point out two errors in the calculation of the ionic product, $Ca^{++} \times CO_3''$, in the previous paper (Smith, 1940*b*). In the first place, the calcium contents there given are uncorrected for strontium, which is usually included in the analysis as calcium. Assuming a calcium/strontium ratio of 30/1 (Lyman & Fleming, 1940), the true calcium content of the water may be found. Secondly, the quantities, carbonate alkalinity, total carbon dioxide, and carbonate ion, were found by reference to Buch's tables (1933). These tables are based on a constant relation between chlorinity and titration alkalinity (Titr. Alk. 0·123 Cl °/₀₀), a relation which holds for most open-ocean sea waters. On the Bahama Bank, however, a marked increase in chlorinity may be associated with a considerable decrease in titration alkalinity. For this reason Buch's tables are not applicable to such water, as different values of pK_B', pK_1' and pK_2' must be used in the calculations. When these errors are corrected for, the value of the ionic product, $Ca^{++} \times CO_3''$, becomes $1{\cdot}62 \times 10^{-6}$ at 30° C. and 36 °/₀₀ instead of $1{\cdot}27 \times 10^{-6}$ as previously reported. Similarly, at 20° C. and 36 °/₀₀, $Ca^{++} \times CO_3''$ becomes $1{\cdot}87 \times 10^{-6}$ instead of $1{\cdot}77 \times 10^{-6}$.

EXPERIMENTAL METHOD

The principle adopted for attaining equilibrium was that of shaking sea water in sealed bottles with a quantity of the naturally precipitated bottom deposit, which was washed with distilled water and dried at 120° C.; about 1 g. was added to each bottle. Standard sampling bottles of 500 ml. capacity were used, leaving about 100 ml. air space. The bottles with solid, but without water, were sterilized in an autoclave. Save for four bottles in the first experiment, the sea water was filtered into the bottles through Berkefeld filters. It had previously been ascertained that there was no change in the titration alkalinity of a sample on filtering (cf. Gee, 1932), although the *p*H increased slightly, owing to the escape of carbon dioxide from solution under reduced pressure.

The bottles were then mounted in a constant temperature shaking apparatus, which was maintained at above or below air temperature, constant to ± 0·2° C. Bottles were removed from time to time and estimations of the *p*H exponent, titration alkalinity, calcium content, and chlorinity, were made. On removing a bottle from the shaker, it was left in the thermostat for 24 hr. to settle; 10 ml. was then run into a test-tube, and the *p*H estimated colorimetrically in the usual way with cresol red. The remainder of the sample was filtered, and the titration alkalinity, calcium content and chlorinity then estimated. In the calculations which were necessary to obtain these values use was made of various constants, such as K_1', K_2' and K_B'. The constants K_1' and K_2' represent the stoichiometric dissociation constants of carbonic acid in sea water, and they are defined by Wattenberg (1933). K_B' represents the stoichiometric dissociation constant of boric acid in sea water as defined by Buch (1933). The relation between *p*H and the activity of the hydrogen ions (a_{H}) is also defined by the same two authors.

The following analytical methods were employed:

(1) *Hydrogen-ion concentration.* Palitzsch boric acid-borate buffers were used for making up the standard buffer tubes, which covered the range at *p*H 0·02 interval. The *p*H in situ ($p\mathrm{H}_{\mathrm{W}}$) was obtained by applying the corrections given by Buch (1937).

(2) *Titration alkalinity* was estimated in duplicate by Wattenberg's (1930) method.

(3) *Calcium content.* Kirk and Schmidt's double precipitation method as adapted for sea water by Kirk & Moberg (1933) was used. More reliable results could thus be obtained than by Gripenberg's (1937) single precipitation method. Estimations were made in duplicate, and the maximum deviation from the mean was 2·0 mg./l., or 0·05 m. mol./l.

(4) *Chlorinity* was determined by Knudsen's standard method.

(5) *Carbonate alkalinity* was calculated directly from the titration alkalinity and *p*H by means of the equations

$$\text{Carb. Alk.} = \text{Titr. Alk.}\ \frac{K_B' C_{\Sigma \mathrm{H_3BO_3}}}{a_{\mathrm{H}^+} + K_B'}, \tag{i}$$

$$C_{\Sigma \mathrm{H_3BO_3}} = 0{\cdot}0225\ \mathrm{Cl}\ {}^0\!/_{00}\ (\text{m. mol.}) \quad (\text{Buch, 1933}). \tag{ii}$$

Values of pK_B' were taken from the table given by Buch (1933).

(6) *Total carbon dioxide*, $\Sigma \mathrm{CO_2}$, was calculated from the equation derived by Moberg *et al.* (1934) from the equilibria equations for the carbonic acid system:

$$\Sigma \mathrm{CO_2} = \frac{\text{Carb. Alk.}}{1 + (2K_2'/a_{\mathrm{H}^+})}\left(1 + \frac{a_{\mathrm{H}^+}}{K_1'} + \frac{K_2'}{a_{\mathrm{H}^+}}\right).$$

Values of K_2' were taken from Buch (1933), and of K_1' from Wattenberg (1933).

(7) *Carbonate ion*, $C_{CO_3''}$, was similarly calculated directly from the equation

$$C_{CO_3''} = \frac{K_2'}{a_{H^+}} \frac{\text{Carb. Alk.}}{1+(2K_2'/a_{H^+})}.$$

EXPERIMENTAL RESULTS

The first experiment was made with water collected 22 miles due north from Williams Cay on the central part of the Bank (cf. Smith (1940 *a*) for general description of the Great Bahama Bank). Ten bottles of water were collected together with a sample of the bottom deposit. Four of these were prepared as described above, while a second four were placed in the shaker without previous filtration through Berkefeld filters. These were subsequently removed in pairs, one unfiltered and one filtered sample, at intervals of 11, 24, 33 and 38 days. The temperature was 29·7 ± 0·2° C. One sample of sea water was analysed as an initial control, while a second was allowed to stand for 19 days before analysis. The analyses are shown in Table I.

The most striking feature of these results is the continuous increase in titration alkalinity, calcium content and total carbon dioxide in all the bottles. Closer inspection of these figures, however, reveals that while the gain in calcium and titration alkalinity is practically identical (within the limits of error of the calcium estimation), the total carbon dioxide content has increased more rapidly. One must conclude, therefore, that carbon dioxide has been produced in the bottles by biological activity. Owing to the fact that there was only a slight gain in total carbon dioxide content when sea water alone was allowed to stand for 19 days, it is more probable that the source of the marked increase in the shaken bottles was due to the added solid. The effect of this increasing carbon dioxide content was to depress the *p*H and consequently the carbonate-ion content to such an extent that the water became unsaturated with respect to calcium carbonate. This caused calcium carbonate to be dissolved from the solid phase as shown by the increasing carbonate alkalinity and calcium content. As each analysis is based on a separate bottle of water, the results cannot be regarded as a progressive series, and considerable variation in the final state is to be expected. The column in Table I showing values of the ionic product, $Ca^{++} \times CO_3''$, corrected to 36 ‰ S, shows that, despite considerable variation in *p*H and carbonate alkalinity, this quantity is practically a constant. The fact that the calcium carbonate in solution has increased in all bottles precludes the possibility of this value of the ionic product representing an over-saturated condition, while its constancy within such narrow limits is indicative of conditions close to the true equilibrium between the solid and liquid phases. This experiment shows, also, that there is no systematic difference in the behaviour of the filtered and unfiltered water, and in subsequent experiments filtered water only was used.

TABLE I. EXPERIMENT I. THE SOLUBILITY PRODUCT CONSTANT, K'_{CaCO_3} IN SEA WATER. WATER FROM THE GREAT BAHAMA BANK, 22 MILES N.N.W. FROM WILLIAMS CAY, SHAKEN WITH THE NATURAL BOTTOM DEPOSIT. TEMPERATURE = 29·7 ± 0·2° C.

No.	Date of analysis	Description	S °/oo	pHw	Tit. Alk. m. eq./l.	$C_{Ca^{++}}$ m. mol./l.	Carb. Alk. m. eq./l.	ΣCO_2 m. mol./l.	$C_{CO_3''}$ m. mol./l.	$Ca^{++} \times CO_3'' \times 10^6$ at 36 °/oo
10	18. vii. 40	Experiment started not shaken, no solid added	36·18	8·15	1·781	10·35	1·671	1·445	0·221	2·27
12	6. viii. 40	Not shaken, no solid added, stored 19 days	36·26	8·08	1·779	10·36	1·682	1·489	0·198	2·03
1	30. vii. 40	Unfiltered. Shaken with solid 11 days	36·22	7·79	1·786	10·48	1·729	1·648	0·120	1·24
2	13. viii. 40	Unfiltered. Shaken with solid 24 days	36·37	7·70	1·921	10·41	1·875	1·808	0·108	1·09
3	21. viii. 40	Unfiltered. Shaken with solid 33 days	36·43	7·69	2·022	10·42	1·977	1·905	0·111	1·13
4	26. viii. 40	Unfiltered. Shaken with solid 38 days	36·42	7·69	1·943	10·45	1·898	1·829	0·107	1·09
5	30. vii. 40	Filtered. Shaken with solid 11 days	35·88	7·73	1·905	10·40	1·856	1·775	0·113	1·18
6	13. viii. 40	Filtered. Shaken with solid 24 days	36·32	7·65	2·269	10·56	2·230	2·164	0·115	1·19
7	21. viii. 40	Filtered. Shaken with solid 33 days	36·29	7·72	2·048	10·43	2·000	1·919	0·120	1·23
8	26. viii. 40	Filtered. Shaken with solid 38 days	36·08	7·72	1·880	10·35	1·832	1·758	0·110	1·14

TABLE II. EXPERIMENT II. THE SOLUBILITY PRODUCT CONSTANT, K'_{CaCO_3} IN SEA WATER. COMPOSITE WATER SAMPLE FROM THE GREAT BAHAMA BANK, SHAKEN WITH THE NATURAL BOTTOM DEPOSIT.
TEMPERATURE = 29·7 ± 0·2° C.

No.	Date of analysis	Description	S ‰	*p*Hw	Tit. Alk. m. eq./l.	$C_{Ca^{++}}$ m. mol./l.	Carb. Alk. m. eq./l.	ΣCO_2 m. mol./l.	$C_{CO_3''}$ m. mol./l.	$Ca^{++} \times CO_3''$ $\times 10^{-6}$ at 36 ‰
C	6. viii. 40	Not shaken and no solid added	38·22	8·05	2·105	11·01	2·003	1·781	0·228	2·25
A	19. viii. 40	Filtered. Shaken with 1 g. solid 14 days	37·79	7·71	1·946	10·84	1·895	1·816	0·113	1·12
B	19. viii. 40	Filtered. Shaken with 1 g. solid 14 days	37·83	7·73	1·957	10·80	1·903	1·821	0·119	1·17

TABLE III. EXPERIMENT III. OPEN-OCEAN SEA WATER SHAKEN WITH BOTTOM DEPOSIT FROM THE GREAT BAHAMA BANK.
TEMPERATURE = 29·7 ± 0·2° C.

No.	Date of analysis	Description	S ‰	*p*Hw	Tit. Alk. m. eq./l.	$C_{Ca^{++}}$ m. mol./l.	Carb. Alk. m. eq./l.	ΣCO_2 m. mol./l.	$C_{CO_3''}$ m. mol./l.	$Ca^{++} \times CO_3''$ $\times 10^{-6}$ at 36 ‰
F	4. ix. 40	Open-ocean water. Not shaken and no solid added	36·33	8·24	2·495	10·67	2·367	1·989	0·362	3·80
A	9. ix. 40	Filtered. Shaken with 1 g. solid 5 days	36·20	7·88	2·120	10·41	2·053	1·919	0·168	1·74
B	9. ix. 40	Filtered. Shaken with 1 g. solid 5 days	36·22	7·90	2·136	10·44	2·067	1·905	0·175	1·82
C	19. ix. 40	Filtered. Shaken with 1 g. solid 12 days	36·13	7·80	2·065	10·40	2·008	1·901	0·141	1·46
D	21. ix. 40	Filtered. Shaken with 1 g. solid 16 days	36·17	7·79	2·022	10·39	1·966	1·872	0·136	1·40
E	21. ix. 40	Unfiltered. Shaken without solid 16 days	36·17	8·22	2·491	10·61	2·369	2·002	0·351	3·70

The second experiment was carried out on a small composite sample made up from a number of water samples taken at several different places on the Bahama Bank (Table II). The initial titration alkalinity was higher and the *p*H lower than that of the water in Experiment I. After 14 days' shaking with solid there was a fall in titration alkalinity and *p*H, although as in the previous series the total carbon dioxide content increased. In these samples, therefore, the predominant process has been precipitation of calcium carbonate despite the liberation of carbon dioxide by some other agency. At the end of the experiment the value of the product, $Ca^{++} \times CO_3''$, lies well within the limits of variation found in Experiment I. The average value of the ionic product based on the ten observations made in these two experiments is $1{\cdot}16 \pm 0{\cdot}05 \times 10^{-6}$. This figure is put forward as a new value for the solubility product constant (K'_{CaCO_3}) of calcium carbonate in sea water at 30° C. and 36 °/oo.

A third experiment in which open-ocean sea water was shaken with added solid was made to check the previous results. The data (Table III) show that at the start of the experiment the water had the normal characteristics of ocean water in these latitudes. On shaking with solid there was a marked fall in titration alkalinity, *p*H, and total carbon dioxide content, which was continuous to the end of the experiment. Comparison of the amounts of calcium carbonate precipitated as shown by the reduction in carbonate alkalinity and calcium content, and the associated decrease in total carbon dioxide again show that there has been a slight carbon dioxide production by biological activity. After 16 days' shaking the ionic product, $Ca^{++} \times CO_3''$, had fallen to $1{\cdot}40 \times 10^{-6}$, indicating that final equilibrium had probably not been attained, though the water at that point is only 20 % supersaturated. From this experiment it is apparent that equilibrium is more readily attained when sea water is used which has precipitated the greater part of its excess calcium carbonate under natural conditions. The two samples F and E in Table III show the effect of shaking sea water for 16 days with no solid phase present. On correcting for the slight difference in chlorinity, it can be seen that the carbonate alkalinity and total carbon dioxide have increased slightly. At least a part of the rise in carbonate alkalinity may be attributed to the solvent action of the water on the glass bottle, but the increase is so small that it would have no significant effect on the product $Ca^{++} \times CO_3''$.

DISCUSSION

The behaviour of the open-ocean water on shaking with the calcareous bottom deposit in Experiment III follows very closely the pattern observed under natural conditions when ocean water flowed across the shallow banks (Smith, 1940*b*). Owing to the continuous diffusion of carbon dioxide from the water on the bank, however, the *p*H does not fall to the same extent as in the sealed bottles, and consequently more calcium carbonate must be precipitated before equilibrium is attained. It is therefore to be expected that a steady state would only be reached under natural conditions after prolonged contact with the solid phase.

Values for the solubility product constant of calcium carbonate in sea water have previously been put forward by Revelle & Fleming (1934) and Wattenberg & Timmermann (1936). Revelle & Fleming concluded that tropical sea water could only be slightly supersaturated with calcium carbonate. This view has already been shown by the writer to be untenable by direct observations on the Bahama Bank, and the present work confirms this view. Wattenberg & Timmermann, however, found a value of $0{\cdot}52 \times 10^{-6}$ for $K'_{\mathrm{CaCO_3}}$ at 30° C. and 36 ‰. Such a value would suggest that tropical ocean water is as much as 700 % saturated with calcium carbonate. Even the lowest values observed on the Bahama Bank after extensive precipitation has taken place would represent approximately 300 % saturation. Considering the conditions under which the present experiments were conducted, it is difficult to see how the value of $K'_{\mathrm{CaCO_3}}$ can lie below $1{\cdot}16 \times 10^{-6}$. Owing to the fortuitous production of carbon dioxide in the bottles, the water in Experiment I became unsaturated with respect to calcium carbonate, and it was in fact being dissolved from the solid phase. In this case if the average value from these observations does not represent the equilibrium, it should be too low and not too high. In Experiment III, however, the ocean water was still slowly depositing calcium carbonate when the ionic product, $\mathrm{Ca^{++}} \times \mathrm{CO_3''}$, had fallen to the neighbourhood of $1{\cdot}40 \times 10^{-6}$. On this evidence it is suggested that the true value of the solubility product lies very close to $1{\cdot}16 \times 10^{-6}$ under the given conditions of salinity and temperature. Combining such a value with the observations made on the Great Bahama Bank in 1939 shows that the water was in some cases only 140 % saturated with calcium carbonate, which is a more probable figure than 300 %. It is possible that differences in the material used as the solid phase may account for some of the discrepancies in the recorded values of $K'_{\mathrm{CaCO_3}}$. The material used in the present work contains large numbers of minute aragonite needles. Mechanical analyses of similar bottom samples made by Vaughan (1918) show that these deposits contain up to 57·6 % of clay and silt-sized particles (0·05–0 mm. diameter).

It is interesting to note that Gee *et al.* (1932) carried out experiments in which sea water was shaken with solid calcium carbonate in sealed bottles. Calcium content, *p*H, and total carbon dioxide content were estimated after periods of shaking up to 17 days. A very marked fall in *p*H was observed, associated with a reduction of total carbon dioxide and a slight fall in calcium content. From the total carbon dioxide and *p*H values observed by these authors it is possible to compute the carbonate alkalinity and hence the carbonate-ion content by the following equation:

$$\text{Carb. Alk.} = \frac{\Sigma\mathrm{CO_2} \,.\, 1 + (2K_2'/a_{\mathrm{H}^+})}{1 + (a_{\mathrm{H}^+}/K_1') + (K_2'/a_{\mathrm{H}^+})}.$$

In one experiment the results for five bottles which had been shaken for periods of from 5 to 17 days give an average value for $K'_{\mathrm{CaCO_3}}$ of $1{\cdot}10 \times 10^{-6}$ at 30° C. and 36 °/₀₀ when recalculated in this way. Thus this work may be regarded as providing close confirmation of the value found by the writer.

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THE FECUNDITY OF *OSTREA EDULIS*

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(Text-figs. 1–4)

Introduction and Review of Previous Work

Galtsoff (1930) has shown that females of the American oyster (*Ostrea virginica*) may discharge from 15 million to 114 million eggs in a single spawning period, while even after such heavy spawning the gonad still contains a large quantity of eggs. A female of *Ostrea gigas* was shown by the same writer to be capable of producing up to 92 million eggs. These oysters both belong to the group of large oviparous species, which Ranson (1938) and Nelson (1938) have proposed should be separated from the so-called flat oysters under the generic name of *Gryphaea*, with the Portuguese oyster *Gryphaea angulata* as the type. Nelson (1938) and Elsey (1935) have shown that in addition to their fundamental difference in process of reproduction the oviparous and viviparous types differ considerably in the relations between the mantle and the visceral mass. The differences between the two types appear to the writer to warrant the adoption of the two generic names as suggested. The viviparous oysters of the genus *Ostrea* are usually much smaller than the oviparous species, and those which have been investigated have been found to produce far fewer eggs, generally discharging the gonad completely in one spawning act. The earliest known estimate of the number of embryos produced by a European oyster is that of Eyton (1858), who arrived at the figure 1,800,000 for very large oysters. Buckland in 1865 (quoted by Philpots, 1890) stated that he had examined several native oysters of average size and weight, and had never found 'the highest number of spat to be more than 829,655 and the lowest 276,555'; his method of estimation is not recorded. Moebius (1883), by weighing separately the total broods of five individuals and also a small fraction of each, and by counting the embryos contained in the small fraction, arrived at the conclusion that adult females of *O. edulis* produced on the average a brood of 1,012,955 embryos. It is probable that Moebius was dealing with what would be considered to-day to be very large oysters. This estimate has been extensively quoted, for no further detailed observations of the production of adult oysters have since been published. Dantan (1913) has, however, published details of a series of counts of the broods of 1-, 2- and 3-year-old oysters from Arcachon and Brittany, in France. Dantan employed a satisfactory method of counting aliquot parts and gives figures for the broods of six 1-year-old, three 2-year-old and eight 3-year-old oysters; his mean values are 95,500, 247,000 and 725,000 respectively. Gaarder & Bjerkan (1934) also give figures for 1-, 2- and 3-year-old oysters and also for older oysters, viz. 100,000, 250,000, 800,000 and about 1,000,000 respectively, but no details are given of the method of arriving at these figures, or of the number of broods counted. It is not clear whether these represent new estimates or are merely a quotation of the work of Dantan and Moebius. Orton (1937) states that a 1-year-old native oyster from Essex was found to be carrying a brood of about 240,000, while a small 3- or 4-year-old oyster, also from Essex, carried 525,000.

The only other species of flat oyster of which the size of brood is known is *O. lurida*, for which Hopkins (1937) has made some careful observations. Hopkins concludes that the average market-size individual of this species, i.e. one about 5 cm. across the shell, produces a brood of from 250,000 to 300,000 larvae. This figure is the result of examining, and estimating by counting aliquot parts of the whole brood, the broods of twenty-five individuals. Hopkins found that the range of variation was considerable, the smallest brood he found being only 69,490 while the largest was 355,500.

In the course of a study of the sexual cycle and breeding in 1- and 2-year-old oysters, the writer found several with broods of embryos, and it was thought worth while to preserve these broods and to make estimates of the numbers present, inasmuch as the age of the parent oysters was known with certainty, for they had been reared from spat setting on limed tiles in the breeding tanks at Conway, N. Wales (see Cole, 1938). As Dantan (1913) points out, it is especially desirable in work of this kind to have oysters other than native oysters from the natural beds, the age of which is always uncertain. The work of estimating the fecundity of oysters in their first few seasons was later extended, as it became necessary to determine the average size of the brood produced by adult oysters kept under tank conditions at Conway, and to compare this figure with the productivity of different types of oysters from the natural beds.

MATERIAL AND METHODS

The material from which our own observations are drawn consists of several distinct categories of oysters.

In the first place there are groups of 1-, 2- and 3-year-old oysters from our relaying ground in the Menai Straits, all of which had settled on tiles in the Conway tanks before being transferred to the Menai Straits.

Secondly, there is a group of young oysters from the Helford River, Cornwall, the age of which could be determined with more or less certainty, comprising both French relaid oysters of 3 and 4 years, and a number of small native brood oysters whose age was believed to be 2 years. It is possible, however, that one or two of the latter may have been 3 years old.

Thirdly, there is a group of large oysters taken from those used as breeding stock in the Conway tanks, and obtained by us from the Yealm River, S. Devon. The majority of these oysters originated in France, being laid down for a year or more in the Yealm River before dispatch to Conway.

Fourthly, there are two groups of large oysters from the Helford River, Cornwall, one consisting of those native to the river, the other comprising Brittany oysters, which are laid down annually in large quantities in the Helford River.

The following method was adopted in determining the number of embryos comprising a brood. The embryos were carefully washed free from the gills, palps and mantle of a gravid oyster, and were killed with formalin. They were then collected by pipette and placed in a round-bottomed flask or a beaker, and the volume made up to a fixed amount, either 250 or 400 c.c. The contents of the flask or beaker were thoroughly agitated and five, or on some occasions ten, exactly determined samples were removed with an automatic pipette. The pipettes used delivered 0·52 and 0·45 c.c. respectively. The embryos contained in each of these aliquot parts were counted under a binocular microscope on a squared counting plate and a mean value determined; multiplied by the appropriate factor, this gave a figure for the total brood. At the same time, the colour, to the naked eye, of the brood as a whole and the stage of development reached by the embryos were noted. Where oysters were dredged from the beds they were opened as quickly as possible, as abortion of embryos may occur through rough handling.

OBSERVATIONS

One-year-old oysters. Gerbe (1876) was the first to observe that oysters may produce eggs even in the first year of their life, i.e. in the summer following that in which they attached themselves. He found thirty-five with embryos out of a total of 435 1-year-old oysters from Arcachon. This observation was followed by the work of de Lacaze-Duthiers (1893), who, after several years' experience of oysters held in the aquaria of the biological station at Roscoff, concluded that only a very few oysters bred during their second and third years, and then only to a slight extent, and that reproduction was not fully assured until their fourth year. Gerbe's work has, however, since been supported by Dantan (1913), who found that of 133 1-year-old oysters from Arcachon, opened on 8 August, six contained broods of embryos. There is good reason for believing that Dantan was able to determine exactly the age of the material with which he was dealing, always a difficult matter unless the oysters have been reared on tiles or have been under observation since settlement. Orton (1922) has recently recorded also that of oysters settling in 1920 in English waters, certain of the larger individuals liberated larvae in the summer of 1921, but the same author later states (Orton, 1937) that in his opinion this state of affairs is not usual in England 'where young oysters may not usually produce the first batch of eggs until their third or even fourth summer'. Work which I have at present in hand shows that the proportion of young oysters functioning as females in the summer following attachment may in some years be larger than was formerly suspected.

Dantan was fortunate in obtaining six 1-year-old oysters with broods of embryos, the mean number in a brood being 95,500 with a range of from 69,000 to 144,000. The average length of the shell of these oysters was 3·4 cm. I have unfortunately only opened one such oyster which was actually carrying a brood of embryos, although ripe females have been of fairly frequent occurrence. This one oyster measured 3·8 cm., and carried a brood of 91,628 when opened on 25 October 1938. The embryos were in the late gastrula stage.

TABLE I. BROODS OF EMBRYOS OBTAINED FROM 2-YEAR-OLD OYSTERS FROM THE MENAI STRAITS, NORTH WALES

Date	No. of embryos	Stage of development of embryos	Colour
29. vi. 38	432,200	Trochophores	White
29. vi. 38	320,900	Trochophores	White
29. vi. 38	272,400	Shelled veligers: 0·15–0·165 mm.	White
29. vi. 38	259,100	Gastrulae	White
29. vii. 38	270,900	Trochophores	White
29. vii. 38	189,300	Shelled veligers: 0·15–0·165 mm.	Light grey
Average	290,800		

TABLE II. BROODS OF EMBRYOS OBTAINED FROM SUPPOSED 2-YEAR-OLD OYSTERS FROM THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
2. viii. 39	5·0	257,800	Shelled veligers: 6 % 0·14 mm., 76 % 0·15 mm., 18 % 0·16 mm.	Light grey
16. viii. 39	4·5	108,800	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
16. viii. 39	5·3	161,300	Trochophores	White
21. viii. 39	4·7	41,900	Shelled veligers: 18 % 0·14 mm., 38 % 0·15 mm., 36 % 0·16 mm., 8 % 0·17 mm.	Light grey
1. ix. 39	4·1	84,400	Shelled veligers: 20 % 0·16 mm., 66 % 0·17 mm., 14 % 0·18 mm.	Medium grey
Average	4·72	130,800		

Two-year-old oysters. During 1938 small samples of 2-year-old oysters from our relaying ground in the Menai Straits were opened at monthly intervals from June to September, and from twenty-six oysters opened on 29 June four were found carrying broods, while a further lot of twelve oysters opened on 29 July yielded two more with broods of embryos; the details of these broods are set out in Table I. Those lots of thirty-one and thirteen oysters opened in August and September respectively gave none with broods. All these oysters formed part of the crop obtained on tiles during 1936 in the breeding tanks at Conway. They were transferred to the Menai Straits about a month or so after settlement and were detached from the tiles when about 12 months old.

In Table II are shown details of the broods of embryos obtained from a number of small native oysters from the Helford River, Cornwall. From a knowledge of the normal growth rate in this river, it was possible to say with fair certainty that practically all of these oysters were 2 years old, but it is possible that one or two were unusually small 3-year-old oysters.

TABLE III. BROODS OF EMBRYOS OBTAINED FROM 3-YEAR-OLD OYSTERS FROM THE MENAI STRAITS, NORTH WALES

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
19. vii. 39	6·0	374,600	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
19. vii. 39	6·2	601,600	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
19. vii. 39	6·3	519,800	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
22. vii. 39	5·0	440,000	Trochophores	White
22. vii. 39	5·5	416,300	Trochophores	White
22. vii. 39	6·0	349,400	Trochophores	White
22. vii. 39	6·0	562,800	Shelled veligers: 84 % 0·17 mm., 16 % 0·18 mm.	Dark grey
22. vii. 39	6·5	496,100	Shelled veligers: 48 % 0·17 mm., 52 % 0·18 mm.	Dark grey
22. vii. 39	6·5	693,700	Gastrulae	White
22. vii. 39	7·0	555,400	Gastrulae	White
Average	6·1	501,000		

TABLE IV. BROODS OF EMBRYOS OBTAINED FROM 3-YEAR-OLD RELAID BRITTANY OYSTERS FROM THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
1. viii. 39	5·5	482,800	Shelled veligers: 0·15–0·16 mm.	Light grey
1. viii. 39	6·2	275,900	Shelled veligers: 8 % 0·17 mm., 28 % 0·18 mm., 52 % 0·19 mm., 12 % 0·20 mm.	Dark grey
16. viii. 39	5·7	216,200	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
16. viii. 39	6·4	492,200	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
Average	5·95	366,800		

Three-year-old oysters. During 1939 a number of 3-year-old oysters from our relaying ground in the Menai Straits were opened and any broods found were preserved. The details of these broods are shown in Table III. These oysters formed part of the crop obtained in 1936 in the tanks at Conway. Unfortunately it was not possible to collect gravid oysters at different times during the season, as the writer was away from Conway during the greater part of the summer.

A small number of individuals carrying embryos were found among samples of 3-year-old relaid Brittany oysters, opened on the Helford River, Cornwall, during the summer of 1939. The details of these broods are shown in Table IV. These oysters had been obtained in April 1939 from Locmariaquer in the Gulf of Morbihan and were stated to be '2-year olds', i.e. 1936 crop, when bought. There is no reason to doubt that they were correctly described, since they were originally caught on tiles, so that the time of settlement would be known. When opened they were of course in their fourth summer, i.e. they were a month or so over 3 years old.

Table V. Broods of Embryos obtained from Relaid Brittany Oysters, 4 years or more of age, from the Helford River, Cornwall

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
18. vii. 38	7·5	1,163,700	Shelled veligers: 25 % 0·17 mm., 65 % 0·18 mm., 10 % 0·19 mm.	Light grey
19. vii. 38	8·0	567,400	Shelled veligers: 16 % 0·16 mm., 32 % 0·17 mm., 48 % 0·18 mm., 4 % 0·19 mm.	Medium grey
5. viii. 38	6·5	1,393,300	Trochophores	White
5. viii. 38	7·0	181,800	Gastrulae	White
5. viii. 38	9·5	1,730,200	Trochophores	White
8. viii. 38	6·5	1,189,400	Shelled veligers: 30 % 0·16 mm., 70 % 0·165 mm.	Light grey
*5. viii. 39	6·4	1,239,100	Shelled veligers: 26 % 0·16 mm., 74 % 0·17 mm.	Medium grey
Average	7·34	1,066,400		

* 4 years old.

Oysters 4 years old, or older. An oyster when 4 years old has frequently reached marketable size and, for practical purposes, may be regarded as adult. The only oyster actually known to be 4 years old from which I have obtained a brood of embryos was a single relaid Brittany oyster from the Helford River. This individual measured 6·4 cm. across the shell and carried a brood estimated at 1,239,100. This oyster is included in Table V, which also gives details of the broods of a number of relaid Brittany oysters, 4 years or more of age, from the Helford River, Cornwall. It is probable that most of the oysters whose broods are detailed in this table were actually 4 or 5 years old when opened, as Brittany oysters are usually sold from the Helford River at this age.

In addition to those from relaid Brittany oysters, broods of embryos were also obtained from a number of oysters native to the Helford River, and the details of these are shown in Table VI. It is possible that one or two of the smallest of these native oysters may have been only 3 years old, but the majority would be 4 or 5 years of age.

From time to time broods of embryos have been collected from adult oysters used as breeding stock in the Conway tanks. Such oysters are brought into the tanks early in May of each year and are taken out again in September and relaid on our own grounds in the Menai Straits, where they remain until taken up again early in the following May. All were originally obtained from the Yealm River, S. Devon, from which a new consignment is added each year. The majority of these oysters are French oysters from the Gulf of Morbihan, and were laid down in the Yealm River for at least 12 months before being sent to Conway. Details of the broods of embryos obtained are shown in Table VII. The majority of these oysters were probably 5 or 6 years old when opened.

TABLE VI. BROODS OF EMBRYOS OBTAINED FROM ADULT OYSTERS NATIVE TO THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
19. vii. 38	6·0	571,600	Shelled veligers: 20 % 0·165 mm., 10 % 0·17 mm., 60 % 0·18 mm., 10 % 0·19 mm.	Medium grey
27. vii. 38	5·75	944,200	Segmenting eggs and morulae	White
27. vii. 38	6·0	353,100	Trochophores	White
27. vii. 38	7·0	1,075,900	Trochophores	White
27. vii. 38	8·5	1,401,500	Shelled veligers: shell not completely enclosing body, *ca.* 0·15 mm. in length	White
5. viii. 38	8·0	322,200	Gastrulae	White
Average	6·88	778,100		

TABLE VII. BROODS OF EMBRYOS OBTAINED FROM OYSTERS, 4 YEARS OR MORE OF AGE, FROM THE CONWAY BREEDING TANKS

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
17. viii. 38	6·35	1,172,800	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
17. viii. 38	6·5	559,100	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
19. viii. 38	8·0	1,115,800	Shelled veligers: 10 % 0·15 mm., 20 % 0·16 mm., 70 % 0·165 mm.	Black
20. viii. 38	7·0	1,245,800	Shelled veligers: 15 % 0·165 mm., 65 % 0·17 mm., 20 % 0·18 mm.	Black
31. viii. 38	6·7	544,000	Morulae	White
31. viii. 38	7·3	663,600	Early gastrulae	White
8. ix. 38	7·0	588,700	Shelled veligers: 5 % 0·165 mm., 90 % 0·17 mm., 5 % 0·18 mm.	Dark grey
8. ix. 38	7·0	749,000	Shelled veligers: 5 % 0·16 mm., 95 % 0·165 mm.	Dark grey
3. vii. 39	No record	1,091,400	Shelled veligers: 40 % 0·16 mm., 60 % 0·17 mm.	Medium grey
Average	*6·98	858,900		

* Average of eight only.

The data derived from all the above year-groups of oysters are summarized in Table VIII.

TABLE VIII. SUMMARY OF THE DATA FROM ALL BROODS OF EMBRYOS

Age	No.	Mean length of shell cm.	Range of variation in number of embryos	Average number of embryos per brood	Origin of parent oysters
1 year	1	3·8	—	**91,600**	Conway oyster from Menai Straits
2 years	6	No record	189,300–432,200	290,800	Conway oysters from Mena Straits
*2 years	5	4·72	41,900–257,800	130,800	Helford River natives
All 2-year-old oysters	**11**			**218,100**	
3 years	10	6·1	349,400–693,700	501,000	Conway oysters from Menai Straits
3 years	4	5·95	216,200–492,200	366,800	Brittany oysters, relaid Helford River
All 3-year-old oysters	**14**	6·06	—	**462,600**	
4 years	1	6·4	—	1,239,100	Brittany oyster, relaid Helford River
4 years and over	6	7·5	567,400–1,730,200	1,066,400	Brittany oysters, relaid Helford River
4 years and over	6	6·88	181,800–1,401,500	778,100	Helford River natives
4 years and over	9	†6·98	544,000–1,245,800	858,900	Oysters from Conway tanks, mainly relaid Brittanys from Yealm River, S. Devon
All oysters 4 years old and over	**22**	‡7·07		**902,900**	

* Probable age, may have been 3 years old in few cases.
† Average of eight only (see Table VII).
‡ Average of twenty-one only (see Table VII).

DISCUSSION

In Fig. 1 length of shell is correlated with brood strength. It will be seen that the number of embryos produced varies rather widely in oysters of similar age and length of shell. A comparable variation has also been recorded in *Ostrea lurida* by Hopkins (1937). Such a variation may in part be due to the fact that length of shell alone is not a completely satisfactory criterion of reproductive capacity, as in oysters with shells of similar length the volume of the meat and the degree of fatness, upon which the reproductive capacity presumably depends, may vary rather considerably. In recent work on the sexual cycle of young oysters I have adopted the weight of the soft parts as giving a better measure of the reproductive capacity.

This variation in brood size among oysters of similar age is well brought out in Fig. 2, in which the observations have been grouped in year-classes, those observations appertaining to adult oysters being grouped arbitrarily opposite the 5-year mark. This method of treating the data shows that the 2- and 3-year-old oysters apparently belong to more or less homogeneous groups, but the observations of brood size among adult oysters appear to group themselves about two points, and this suggests that we are dealing with two distinct classes of adult oysters varying considerably in brood strength. The reason for this peculiar grouping is not clear, but it may perhaps be due to a considerable reduction in the number of embryos produced by oysters maturing as females in the second half of the season after an early male phase, when compared with individuals which enter on the female phase as soon as the season begins. This possibility is further discussed later in this paper (p. 255).

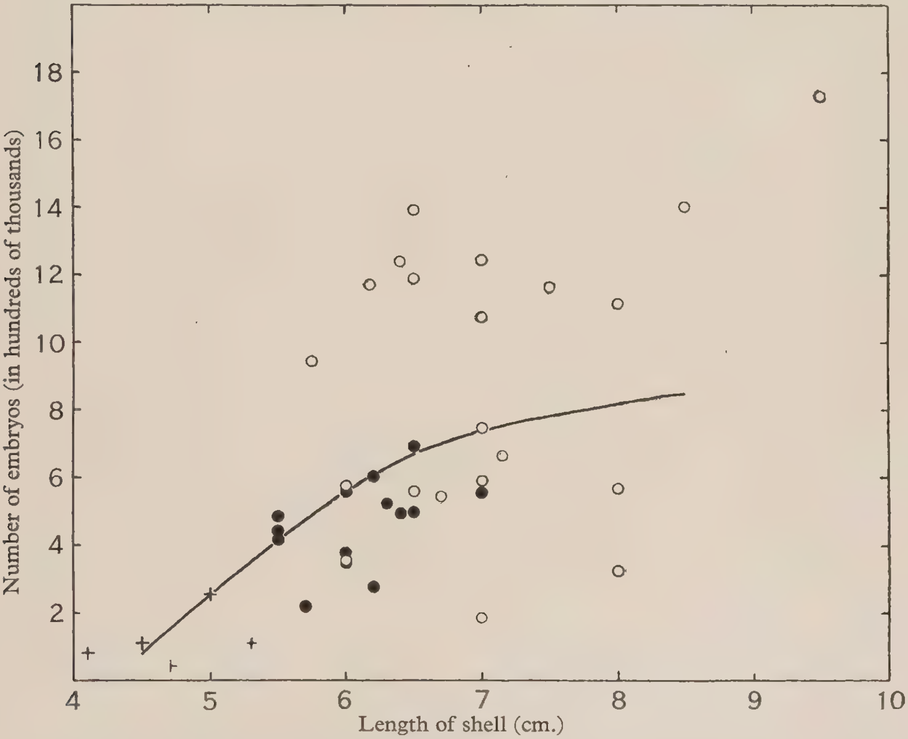

Fig. 1. The relation between length of shell and the number of embryos produced. The curve showing the general trend of the relationship has been drawn through points representing the mean number of embryos per brood in groups of oysters of all ages in the size categories 4·0–4·9, 5·0–5·9, 6·0–6·9 cm., etc. The single observation in the group 9·0–9·9 cm. has been ignored. + = 2-year-old. ● = 3-year-old. ○ = 4-year-old and over.

The curve showing the general relationship between shell length and the number of embryos produced (Fig. 1) has been drawn through points representing the mean brood size of groups of oysters of shell length 4·0–4·9, 5·0–5·9, 6·0–6·9 cm., etc.; the single oyster in the size-group 9·0–9·9 cm. has been omitted. The trend of this curve appears to indicate that it is unlikely that an average brood of more than 1,000,000 will be produced throughout the whole season, even by a population of very large oysters.

The colour to the naked eye of a brood of embryos as it lies in the mantle cavity of the parent oyster is not a very reliable guide to its state of development, for, as can be seen from the tables, certain broods recorded as being light or medium grey in colour contained larger larvae than those which appeared almost black to the naked eye. As anyone who has an extensive experience of oyster larvae will confirm, the degree of pigmentation of individual larvae after liberation is very variable.

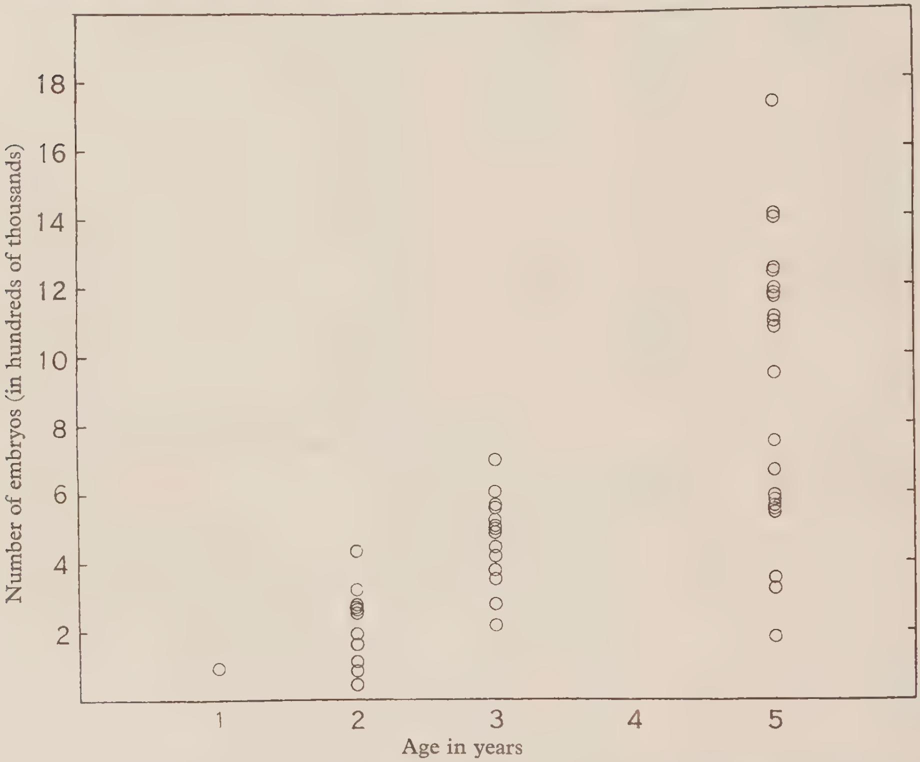

Fig. 2. The relation between the number of embryos produced and the age of the parent oyster. All oysters over 3 years of age have been grouped arbitrarily at 5 years.

The figures given in this paper tend to show that as regards the production of larvae an oyster practically doubles its capacity each year during the first 4 years of its life and that it is only when 4 years old that an oyster may be looked upon as adult, and playing its full rôle in the reproduction of the species. This is in close agreement with the opinion of most practical oystermen who are agreed that a 3-year-old oyster is readily distinguishable as a juvenile, while one 4 years old is not.

The data obtained from 1- and 2-year-old oysters do not differ markedly from those of Dantan (1913) and Gaarder & Bjerkan (1934), if the latter represent separate estimates, but the figure for 3-year old oysters given by these authors seems rather high and approximates to that of many adult oysters.

The data presented above provide a fairly satisfactory answer to the query as to whether breeding oysters held under tank conditions produce larvae as freely as similar oysters living on the natural beds. The oysters from the Conway tanks are themselves mainly relaid oysters from Brittany and are best compared with similar relaid Brittany oysters from the Helford River. The results of such a comparison are slightly in favour of the oysters from the Helford River, with an average brood of 1,066,400, as against 858,900 for those from the Conway tanks, but the average size of the former lot was some 5 mm. greater. Compared with Helford River natives of about the same size, the oysters from the Conway tanks are slightly superior in the production of larvae. We may therefore conclude that the retention of breeding oysters in large tanks such as those at Conway for a period of 4 months does not impair their reproductive capacity, for it has already been shown (Cole, 1939) that the percentage maturing as females under tank conditions may reach or slightly exceed 50 %, the figure arrived at by Orton (1936) working on the natural beds. In making the above estimate (viz. 50 % females) I had assumed that each oyster produced a brood of 1,000,000 embryos; had I employed the average figure of 858,900 arrived at in this paper, the percentage maturing as females would be slightly increased (see Cole, 1939, Table XXI, p. 39).

There is no indication from the figures presented in the tables of any substantial loss or mortality of embryos during the period of development within the mantle cavity, for very large broods of both early embryos and shelled veligers occur in practically all groups. The amount of variation in shell size between the larvae of a brood is itself variable, although in those broods carried by adult oysters the variation rarely exceeds 0·015 mm. In some of the younger oysters the larvae of a brood varied however as much as 0·03 mm., but a marked difference in development between parts of a brood, such as would indicate successive partial spawnings at a few days' interval, was never observed, although Orton (1936), who has examined a great many oysters carrying larvae, records that such a phenomenon is occasionally observed.

Only in one instance were veligers measuring 0·20 mm. across the shell found in the mantle cavity (see Table IV), the maximum size being usually 0·19 mm., although Orton (1927) has recorded larvae up to 0·22 mm. The usual size of larvae when liberated in the Conway tanks is 0·18 or 0·19 mm., but both slightly smaller and slightly larger larvae are not uncommon. Under natural conditions in the Helford River during 1939 I frequently observed larvae as small as 0·15 mm. in the plankton, such larvae appearing very pale.

As can be seen from the tables, those broods which showed most variation in size amongst the veligers occurred principally late in August and more frequently among immature oysters (see Tables III and IV). It has been observed both at Conway and Port Erin (Bruce, Knight & Parke, 1939) that all broods of larvae are not equally viable and that, as a general rule, those broods which show least variation in size amongst the individual larvae show also the best survival under experimental conditions.

A comparison between 2- and 3-year-old oysters bred in the tanks at Conway and oysters of similar age from the Helford River shows the Conway oysters to be distinctly in advance, and is an indication of the viability and general soundness of oysters bred under enclosed conditions. In making this comparison it should not be forgotten, however, that the oysters from the Helford River were collected rather late in the season, during August, and there is a possibility that such late breeding oysters may produce smaller broods than those breeding during June and July. In Fig. 3 the observations have been grouped according to the month in which they occur and curves

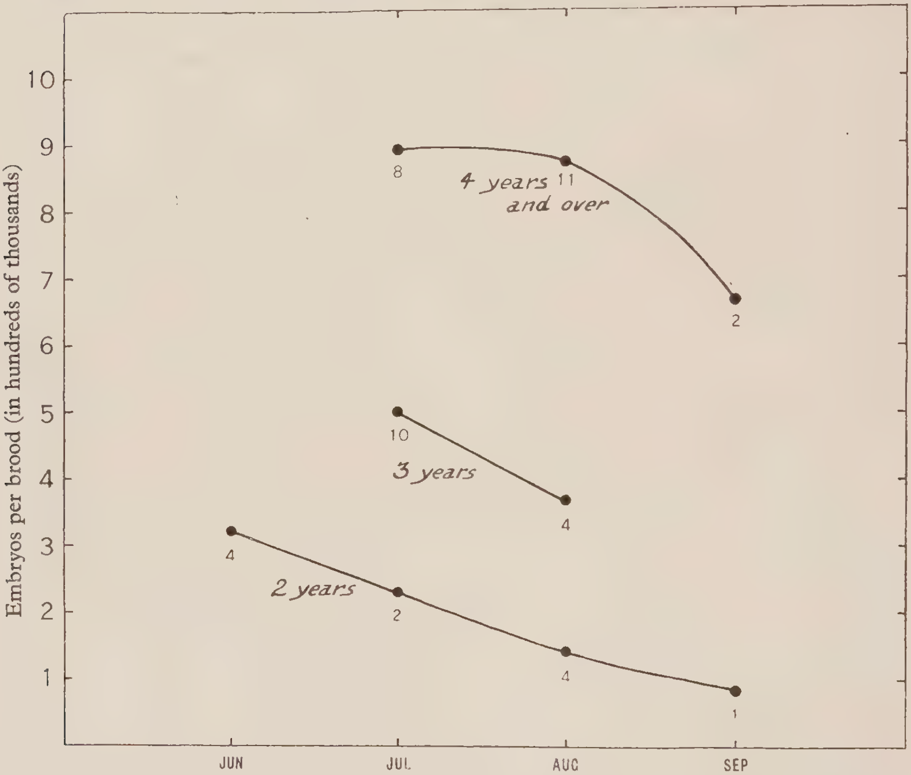

Fig. 3. The variation in the mean number of embryos produced per oyster as the season advances. The observations have been grouped according to the month in which they occur. The figures under the points on the curves indicate the number of observations in each group.

have been drawn through points representing the mean size of brood for each month in the three year-classes of oysters. The single adult oyster with an unusually large brood has been omitted, as its inclusion produced an abnormal displacement of the mean. All three curves fall more or less sharply in the second half of the season and, although the number of observations is small, it suggests that in oysters of all ages there is a general falling off in the number of embryos in a brood as the season advances.

The peculiar grouping of the observations of brood strength in adult oysters, as shown in Fig. 2, strongly suggests that we are dealing with two distinct categories of female spawners differing considerably in brood strength. By taking the mean of each apparent group of observations, the figures obtained for the average number of embryos produced by oysters in the two classes are 1,230,000 and 510,000 respectively. Taken in conjunction with Fig. 3, which shows a marked decline in brood strength as the season advances, these figures suggest that those oysters spawning as females during the second half of the season produce, on the average, rather less than half as many embryos as those spawning earlier. If this is so, and it is recognized that the observations are few, the most likely explanation appears to be that oysters maturing as females after an early male phase have expended a part of their reserves in maturing a crop of sperm, and consequently are only able to ripen a comparatively small batch of eggs, whereas those oysters maturing as females from the outset of the season, without a preliminary male phase, are able to draw upon the whole of the reserves accumulated during the spring.

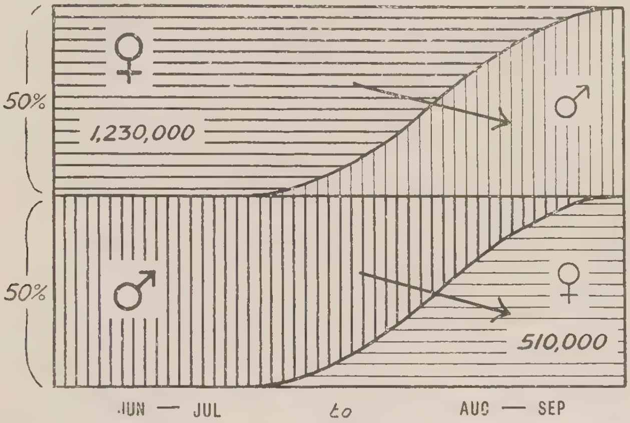

Fig. 4. Diagrammatic representation of the possible effect of sex change during the breeding season on the relative intensities of the male and female phases. The data show the probable mean number of embryos produced by each of the two categories of females.

As the season advances, the percentage of the population spawning either as males or females, without a preceding phase of the other sex, must clearly decline, thus accounting for the shape of the curves in Fig. 3; but it is not possible to fix a definite date after which, for instance, all oysters spawning as females will have experienced a previous male phase early in the season. In unfavourable circumstances, for example in abnormally cold seasons, it is possible that the ripening of those oysters maturing as females from the outset of the season may be delayed until late July or even August. Experience of breeding oysters held in tanks supports this view, for in 1938, for example, although the Conway tanks contained over a thousand oysters no substantial spawning of females occurred until the third week in July (see Cole, 1939).

We arrive therefore at the conclusion that the majority of adult oysters probably experience a sex-change during favourable seasons, and that the early phase, either male or female, is a strong one, quantitatively speaking, and is followed by a very much weaker phase of the other sex. Fig. 4 represents an attempt to give diagrammatic representation to these possibilities. For the purpose of this diagram it has been assumed that each adult oyster experiences a sex-change every season and functions as both male and female each season. The evidence at present available suggests that this occurs only in favourable circumstances, but further investigation is likely to show that the percentage of female spawners is higher than at present supposed. It should perhaps again be stressed that the observations are few and the conclusions are therefore extremely tentative. They are advanced with the particular desire to call attention to the singular grouping of the observations of brood strength among adult oysters, and the interesting lines of inquiry which these observations suggest.

It is interesting to compare the fecundity of adult *Ostrea lurida*, which rarely exceed 5 cm. in diameter, with that of juvenile *O. edulis* of the same shell size. A 3-year-old Conway oyster of 5 cm. (see Table III) gave a brood of 440,000 embryos, while the 2-year-old Conway oysters averaged 290,800 (Table I). Although the sizes of the oysters composing the latter group were not noted at the time, the usual size of oysters of this age is about 5 cm. The broods produced therefore compare favourably with those of adult *O. lurida*, for which the average figure given by Hopkins (1937) is 250,000–300,000. *O. lurida* has, however, a distinct biological advantage for, due principally to the lengthy breeding season, each individual may be expected to mature as a female during each season, while in favourable years the proportion maturing as females *for the second time* may reach 75 % (see Hopkins, 1937).

Now that the practice of selling four grades of oysters, instead of one or two as in former years, seems well established in this country, it is as well to take stock of the effect of this change on the oyster fisheries. On the majority of British oysterages, except those few which still deal exclusively in the true natives, the stock of marketable oysters is maintained to a very large extent by relaying annually in March or April large quantities of imported French oysters. These oysters may in some instances remain on the beds for two or even three or four breeding seasons before being dispatched to market, but a great many are sold during the winter following that in which they are laid down. Those carried forward to another winter season are usually young oysters 2 years and 8 months old when purchased. The contribution made by such relaid oysters from France towards the natural spatfall on our beds is a matter for dispute among practical oystermen. It is possible that the passage from France, and the disturbance which accompanies taking up and replanting during the spring, may so upset the maturation of the gonads of these oysters that they do not breed effectively during the following summer; this point has not yet been fully investigated. It is, however, probable that during subsequent summers spent on English beds they behave in a very similar manner to native oysters. Evidence collected by the writer appears to indicate that this is so. Protagonists of the view that such Brittany relaid oysters do not give rise to any quantity of spat point to the fact that the natural spatfall obtained in rivers where Brittany oysters are laid down shows little, if any, improvement since the practice of importing these oysters began. This is undoubtedly true, but such poor spatfalls may be the result of the operation of a great many adverse factors the nature of which is not at present understood.

Since four grades of oysters are now sold almost everywhere, the beds are practically swept clean every winter of large oysters, those remaining in early spring being mainly very small stunted oysters or young oysters which were '2-year-olds' (actually 2 years and 8 months) when laid down. In March or April the grounds are replanted with a fresh consignment of '2- and 3-year-old' oysters from France, but, as indicated above, the rôle played by these oysters is uncertain. Therefore the stock of acclimatized oysters consists in the main of very small rejected adult oysters and beds of oysters 3 years old, together with such native half-grown oysters as the ground carries. It is therefore of some interest to know to what extent these young oysters may be expected to contribute to the stock of larvae in the rivers and hence to the spatfall. As already noted, the observations given in this paper tend to show that a gravid oyster doubles the number of embryos it produces each year during the first 4 years of its life, and that only when it is 4 years old does it play its full part in the reproduction of the species. We may therefore say that as regards the number of embryos which it produces a 4-year-old oyster is equivalent to at least two 3-year-old or four 2-year-old oysters. The balance is probably in fact more heavily weighted in favour of the use of adult oysters for breeding stock, for it is not yet established that among such 2- and 3-year-old oysters the percentage maturing as females is as high as among adults; the evidence at present available suggests quite the reverse. Although work which I have now in hand tends to show that the percentage of oysters maturing as females in their first year (i.e. their second summer) may, in favourable seasons, be larger than hitherto supposed, yet it is not likely that it will be found to exceed 25 %, and it is probable that on investigation the percentage of 2-year-old oysters maturing as females will be found to be less than 50 %; on the other hand, among well-nourished adult oysters it is likely that more than 50 % of the population will spawn as females in favourable seasons and perhaps, in exceptional circumstances, the whole population. If one assumes that the proportions of female spawners among populations of 2-, 3- and 4-year-old oysters are 40, 60 and 80 % respectively, in any given season, then, taking into account the known differences in brood strength, it is clear that as regards its contribution to the stock of larvae one 4-year-old oyster is equivalent to nearly three 3-year-old, or to eight 2-year-old, oysters. Further investigation is therefore likely to lend additional weight to the view that adult oysters are much to be preferred as breeding stock.

Thus it will be seen that the answer to the question whether it is wise to denude a bed of practically all large oysters each winter will depend very much upon whether the newly relaid Brittany oysters breed effectively during their first summer on English beds. If these oysters can be shown to breed early enough and freely enough to give a crop of spat capable of surviving the winter, then there is no reason for supposing that the removal of the major part of the stock of acclimatized adult oysters is likely to lead to any diminution in the natural spatfall. As mentioned earlier, the breeding of these *freshly laid* oysters has not yet been fully investigated, but the general opinion seems to be that they breed very late in the year on our beds during their first summer and that the spat settling is rather too small to survive in any numbers over the winter.

On beds where French oysters are not laid down, and which at the present day generally carry a rather meagre stock of native oysters, it is probable that the selling of several grades of oysters, resulting as it does in practically clearing the beds of all adult oysters, is definitely detrimental to the continuance of the fishery and the proper replenishment of the stock by the natural spatfall. On such beds it seems advisable to establish a reserve of large oysters for breeding purposes, and to replenish this reserve each year, to the extent of about 20 % of the stock, so as to allow for the annual mortality.

SUMMARY

It is shown that the average number of embryos produced by a gravid oyster is approximately doubled each year until the oyster is 4 years old, when it may be regarded as adult. Estimates have been made of the quantities of embryos produced by one 1-year-old, eleven 2-year-old, fourteen 3-year-old and twenty-two adult oysters; the mean values for these year-classes are 91,600, 218,100, 462,600 and 902,900 respectively.

Since it is probable that the proportion of a population of oysters which matures in the female phase increases progressively up to the age of 4 years, it follows that adult oysters are for this reason superior to 2- or 3-year-old oysters as breeding stock, quite apart from the increased number of embryos produced by each oyster.

As the season advances there appears to be a marked decrease in the average

number of embryos produced by oysters of all ages maturing as females. In adult oysters the observations of brood strength appear to be grouped around two points and this suggests the existence of two distinct categories of adult female-spawning oysters differing considerably in the average number of embryos produced. No such separation into two groups can be observed in the observations of brood strength in 2- and 3-year-old oysters. The average brood strengths of the two classes of adult females appear to be about 1,230,000 and 500,000. It is suggested that this great difference is due to the existence of two types of female spawners, the one maturing as females from the outset of the season, and the other functioning as males early in the season and, following a sex-change, spawning again as females towards the second half of the season. In the first class of females all the reserves accumulated during the spring are available and the batch of eggs ripened is consequently very large, whereas in the second class a part of the reserve materials has already been utilized in maturing an early crop of sperms, with a consequent marked reduction in the number of eggs ripened. The tentative nature of these conclusions is stressed.

In the light of the data obtained concerning the relative fecundity of adult and half-grown oysters, the effect is considered of the practice, now general on British beds, of selling several grades of oysters, thereby depleting the beds of large oysters during the breeding season. It is deduced that on beds where French oysters are relaid each spring little diminution in the natural spatfall is likely to result, provided that it can be shown that these relaid French oysters breed satisfactorily during their first summer on British beds. This matter has not yet been fully investigated. On beds where new stocks of oysters are not laid down each year, it is suggested that a breeding reserve of large oysters should be established if the normal winter marketing operations result in serious depletion of the stock of such large oysters, as undoubtedly often happens on some beds.

ACKNOWLEDGEMENT

In presenting this paper I wish to thank Mr R. E. Savage for his criticism and advice, particularly with regard to the treatment of the data.

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METRIC VARIATIONS IN POPULATIONS OF *CARCINUS MOENAS*

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(Text-figs. 1–8)

INTRODUCTION

In the years 1893, 1895 and 1898 careful measurements were made by Weldon and Thomson (Weldon, 1894, 1898) of the dimensions 'frontal width' and 'carapace length' (Fig. 2) in large numbers of young male *Carcinus moenas* from the beach at Plymouth. From a consideration of these data Weldon found that there was a continuous decrease in the ratio, frontal width/carapace length, with increase in body size, and further that at any particular body size the ratio was progressively smaller in succeeding years. He concluded that natural selection was at work on the population, differentially removing individuals with relatively wide frontal aperture. He suggested that since the building of the breakwater across Plymouth Sound in 1813 the amount of silt in the waters of the Sound had been continuously increasing, so that a relatively wide frontal aperture to the branchial chamber of *Carcinus* became increasingly deleterious to the animal. Weldon thought that his laboratory experiments bore out his conclusions. Both conclusions and experiments have been severely criticized by Cunningham (1928) and others, and there seems little doubt that the criticisms are sound.

The problem of the undoubted decrease in the ratio frontal width/carapace length remained. However, Huxley's demonstration (1932) of the widely occurring phenomenon of differential growth between different dimensions of the same body suggested a possible explanation of the change in the ratio in question, with increase in body size. The graph obtained by plotting frontal width against carapace length on double logarithmic paper (Needham, 1935) showed the straight line characteristic of most cases of differential growth. The graphs for the three years were three parallel straight lines, indicating that differential growth was essentially the same in all. There remained the problem of the vertical separation of the curves, that is, the successive decrease in the initial ratio frontal width/carapace length in succeeding years. The present work, an extension of Weldon's work on natural populations, was intended chiefly as an investigation of this problem. In addition, the suggested explanation of the change in the ratio with increase in body size was tested by measurements of the growth of actual individuals in the laboratory (Fig. 3).

Material and Methods

Three different populations were studied in three successive years, in the neighbourhood of Belfast. It was hoped that by choosing a locality in Larne Lough (Mill Bay) with much silt, one at Greencastle, in Belfast Lough, with a moderate amount of silt, and Ballymacormick, facing the open waters of the Irish Sea and therefore comparatively free of silt, to detect any possible effect of this factor on the dimensions studied. The three localities will subsequently be referred to as L, G, and B respectively (see Fig. 1), followed by '36, '37 or '38 for the three years (1936, 1937, 1938). Samples of male *Carcinus* were collected from the three localities as nearly as possible at the same time each autumn. Orton's observations on the growth of individuals

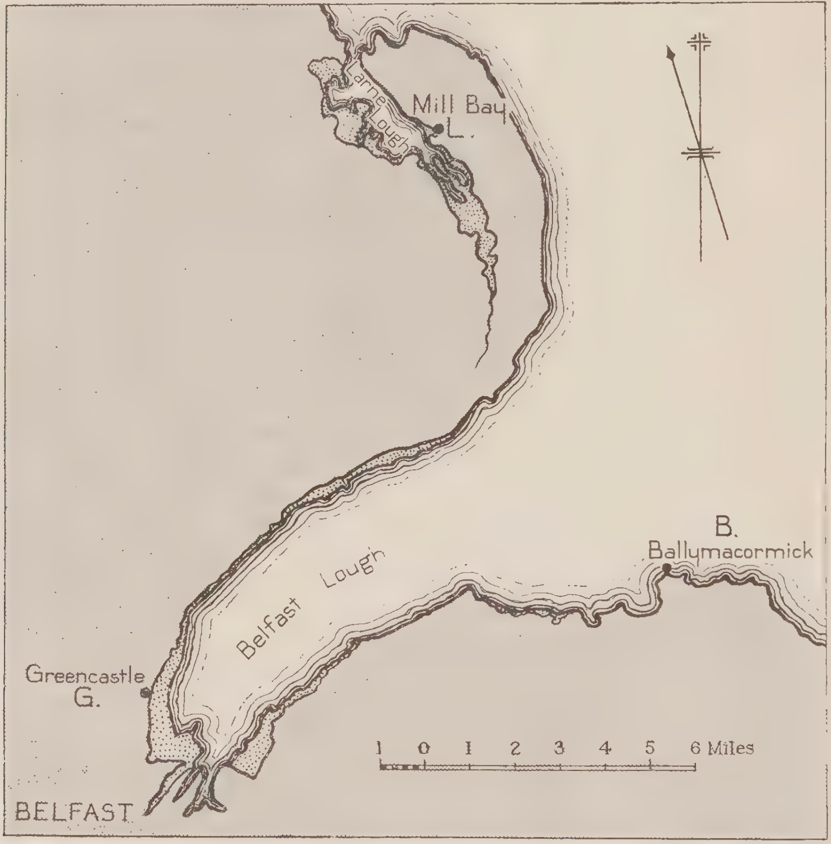

Fig. 1. Outline map of the north-eastern coast of Ireland, in the Belfast district, to show the three localities selected for the study of *Carcinus* populations, and the differences in their situation.

in the field (Orton, 1936) indicated that they reach maturity in a single year, so that the bulk of each sample belonged to one season and was therefore homogeneous in this respect. The samples varied between 180 and 450 in number and covered a range of body size from 4 to 50 mm. Weldon's samples were much larger and covered a much smaller range of body size (10–15 mm.). The same dimensions as those of Weldon were measured (Fig. 2), but 'dentary margin' has not been extensively used in the subsequent analysis. Measurements were made with fine callipers on all individuals above 7 mm. carapace length. The smaller specimens were measured under a binocular microscope with a micrometer eyepiece. The error of measurement is estimated at not more than 1·5 %.

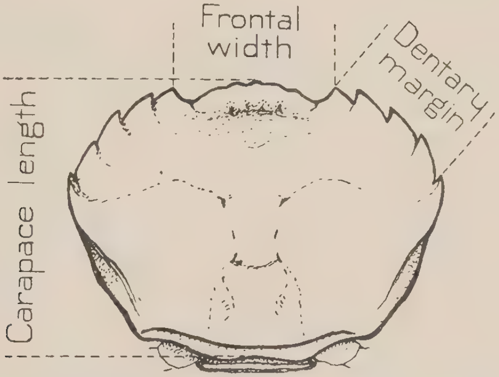

Fig. 2. Outline of carapace of *Carcinus moenas* to show dimensions measured.

The two dimensions, frontal width and dentary margin, were plotted against carapace length on double logarithmic paper. Group points were plotted, the grouping of individuals being at equal intervals on a logarithmic scale, except in the case of G '36 where the grouping was on a linear scale. There appeared to be no essential difference in the resulting graphs, but the logarithmic grouping gives even spacing of the points all along the logarithmic graph.

In the case of frontal width/carapace length the significance of possible differences between populations, in their growth curves, was tested mathematically. The Plymouth graphs (Needham, 1935) had taken the form of parallel straight lines corresponding to the equation of simple allometry (Huxley & Teissier, 1936), $y = bx^{\alpha}$, where α (the slope of the line) is constant in the three years, but b (the initial ratio y/x) decreases in succeeding years. In a case like this it is legitimate to test only for the significance of differences in b and assume that α is quite constant. An appropriate test, originally due to Teissier (1935), has been devised by Reeve and is here applied to the Belfast data.

The data for the nine populations were pooled and plotted on a single graph, double-logarithmically (Fig. 6). From this a mean value of α for all nine, was obtained. Actually it was thought preferable to divide up the data into three sections according to body size, the sections to contain approximately equal numbers of individuals. The sections will subsequently be indicated by the suffixes 1, 2 and 3 to the appropriate population (L '36_1 and so on). Thus the data are divided into twenty-seven sections altogether. In correspondence with the division of the data three consecutive straight lines have been fitted to Fig. 6, their slopes giving the values of α appropriate to the three sections; the probability that α did, in fact, change with increase in body size was the main reason for dividing up the data. Although representing equal numbers of individuals the three sections do not cover equal ranges of body size (Fig. 6).

Using the appropriate mean values of α in each section, the value of b for every individual (about 3500 in all) was calculated from the allometry equation $y=bx^{\alpha}$, the calculation being performed in two steps (x^{α} and then y/x^{α}) using a log-log slide rule. From the series of values of b the mean, $\bar{b}$, was calculated for each of the twenty-seven sections and also the corresponding variance of b (Tables I–III). Any two populations were then considered to be significantly different in any section, if the difference, $\bar{b}_2-\bar{b}_1$, between their mean values of b was greater than twice the standard error of the difference (i.e. more than twice the square root of the sum of their variances of b). The test was applied in two ways, which may be referred to as the general and the detailed tests. In the former, annual differences were tested by pooling the data of the three localities, and local differences in the same way by pooling the data for three years (Table I). This has the advantage of eliminating minor irregularities. In the detailed tests, the data for single populations were compared (Tables II, III). A significant difference is indicated by S and a non-significant difference by 0, together with the sign of the difference. Differences greater than bare significance are marked by more S's, three times the standard error by SS, four times by SSS and so on.

Mr G. M. Spooner has kindly checked the results by an alternative test based on analysis of the variance.

RESULTS AND CONCLUSION FROM THE GRAPHICAL METHODS

Measurements of the growth of individuals show clearly that a decrease in the ratio frontal width/carapace length with increase in body size is manifest in the growth of the individual (p. 261). The data are shown in graphical form in Fig. 3, the short lines joining points representing the dimensions of successive exuviae. The slope of these lines varies around that of the whole population (dotted line), and the individual lines themselves are more or less normally distributed around the population curve. The extent of individual variation is perhaps worth noting. All measurements were carefully made by both workers, and there is little doubt that most of the variation is real. The population curve is a mean curve only (cf. Davenport, 1934; Needham, 1937).

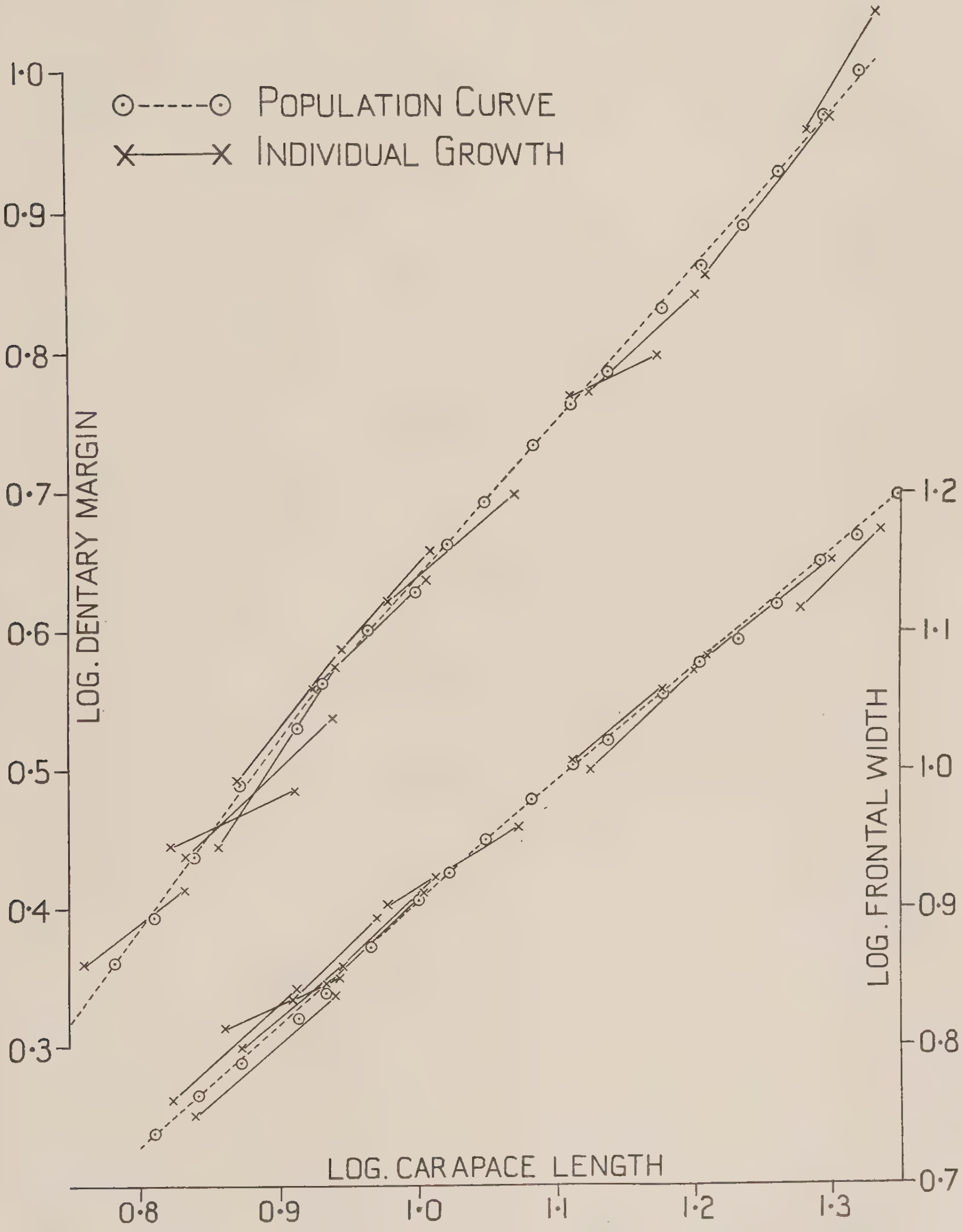

Fig. 3. Growth in frontal width and dentary margin relative to carapace length in individual male *Carcinus* kept in the laboratory, plotted for comparison, on the curve (dotted line) for the whole population in the same year and locality (Greencastle, 1937). Double logarithmic plotting. The lines join dimensions of successive exuviae.

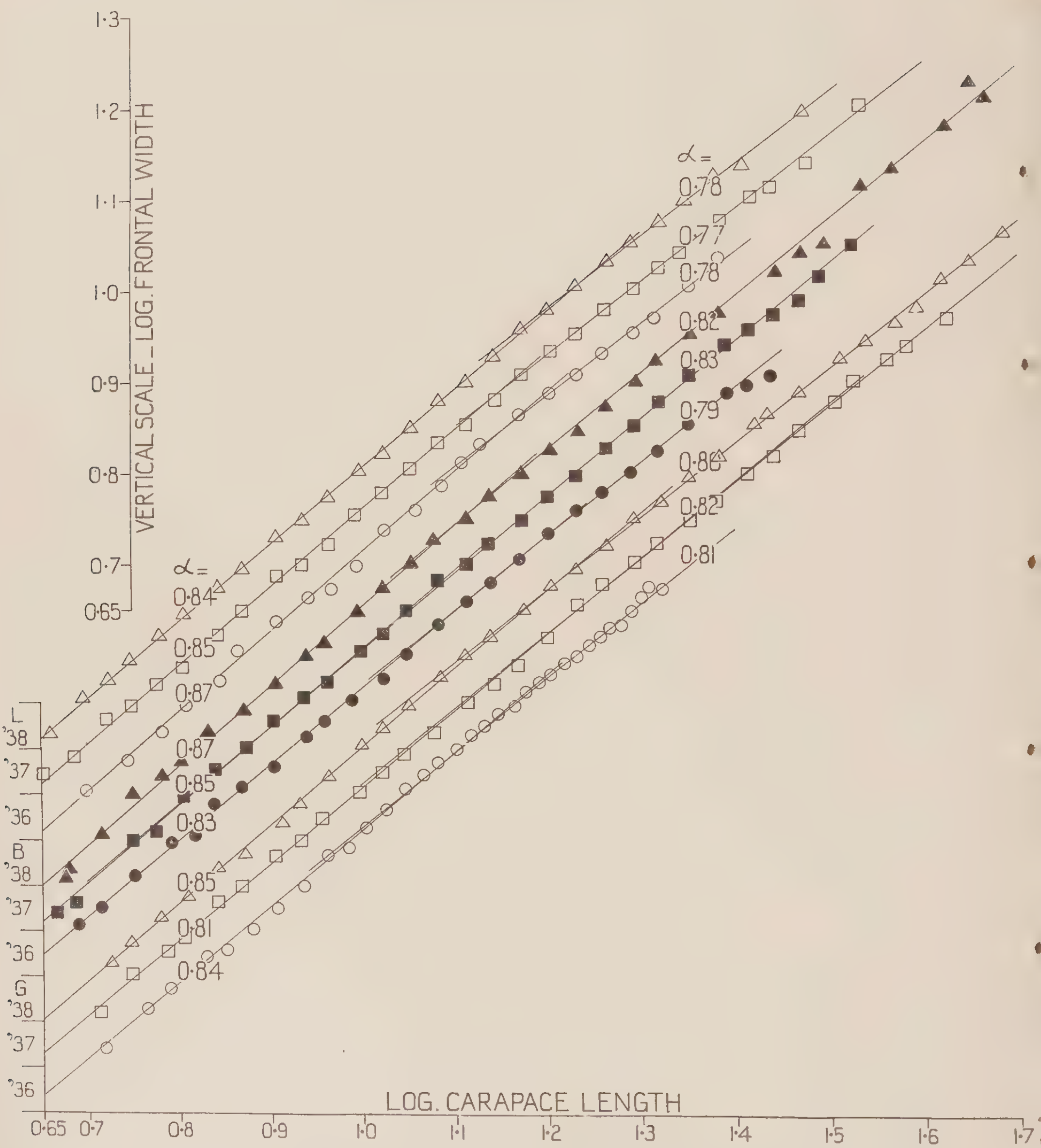

Fig. 4. Growth in frontal width relative to carapace length in nine populations of *Carcinus moenas* (males), from three localities, Greencastle (G), Larne (L), and Ballymacormick (B), in three successive years, 1936, 1937, 1938, to show the differences between the populations. Double logarithmic plotting. The curves are separated out, for clarity, by one large square of the graph paper, in the direction of the ordinate.

The curves of relative growth, frontal width/carapace length, for the Belfast populations (Fig. 4) are essentially similar to the Plymouth curves (Needham, 1935). It is clear, however, that the value of α is not absolutely constant over their much greater range of body size (p. 263), but decreases at larger body sizes. Two consecutive straight lines give reasonable fitting to the points, but it is probable that there is a continuous fall in α and that the three lines of Fig. 6 give a nearer approximation. For the sake of clarity the curves in Fig. 4 have been separated by one large square of the graph paper, in a vertical direction. At G and B there is a clear fall in *b* in '37, '38. The annual decrease is also shown by the wider gap between the curves L '38 and B '36 and between B '38 and G '36 (2-year interval) than between the curves in each locality, spaced at 1-year intervals. The value of α varies

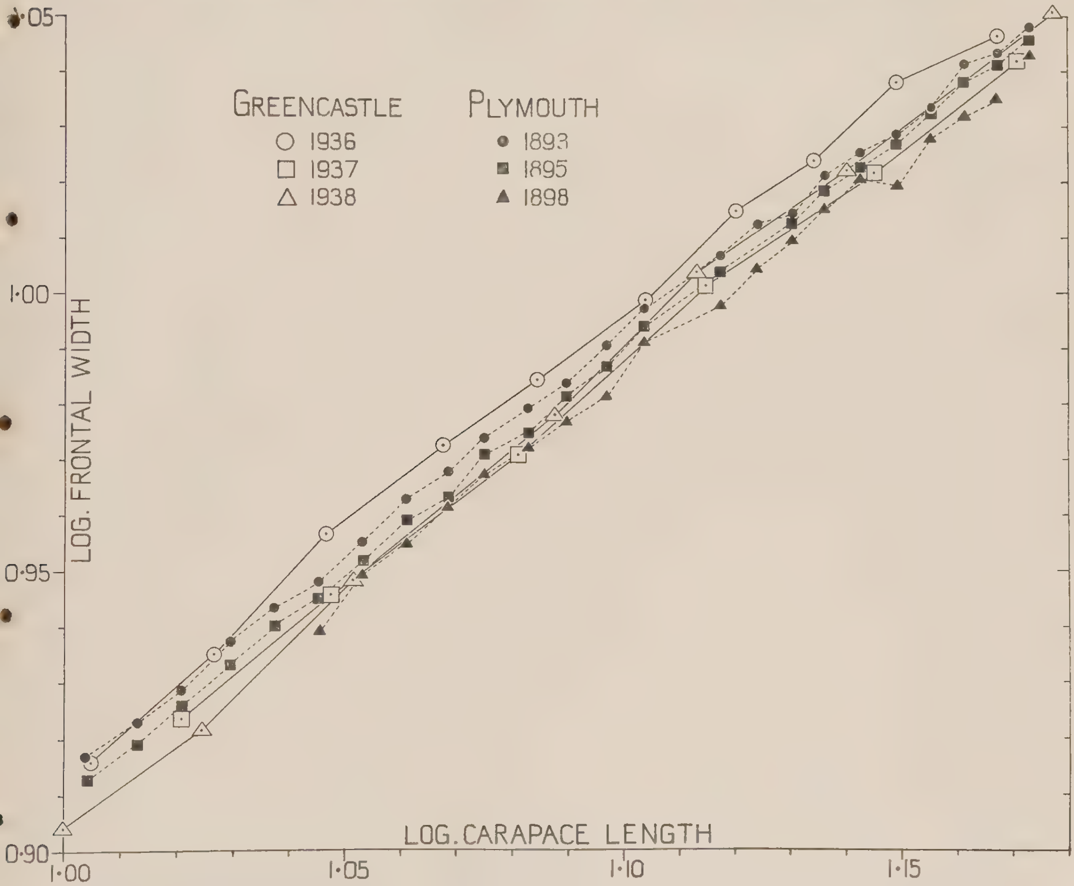

Fig. 5. The population curves for Greencastle male *Carcinus*, 1936, 1937, 1938, superimposed on those for Plymouth males, 1893, 1895, 1898, to show how the two sets overlap, and the parallel annual trend in the two localities. Double logarithmic plotting.

somewhat in the different curves (0·81–0·87 for the first, and 0·77–0·83 for the second section), but an estimate of the standard error for one population (B '36) indicated that variation up to 0·08 was not significant. The division of the curves into two sections prevents a direct comparison with the Plymouth curves, but this has been done graphically in Fig. 5, where the latter are superimposed on the corresponding section of the G curves. The latter are possibly slightly higher than the Plymouth curves (higher value of *b*) and α is also possibly higher, but the differences are much less than the annual differences in the two localities. Since there is an interval of 40 years between the two groups of data it seems probable that the annual trends are not maintained over such long periods. At the same time they were, however, steadily maintained over a 5-year period at Plymouth, and the 2- and 3-year intervals show a correspondingly greater fall in *b* than the 1-year intervals of the Belfast data. The greater scatter among the points on the graphs of the latter is explained by the much smaller samples (p. 263).

Fig. 6 shows clearly that there is a real difference between the different populations in mean frontal width at corresponding body sizes. The grouping by body size was the same for all populations (except G '36, which is very distinct from the rest, therefore), so that the variation in carapace length (horizontal scatter of each group point) is due solely to chance variation in small samples. This variation is very small in the central region of the graph where there were large samples in the group. The variation in frontal width (vertical scatter of the points), on the other hand, shows considerable variation even in this region.

There is an interesting difference between different groups in the extent of this variation in frontal width, even in the central region. It is small in the groups with log carapace length = 1·0, 1·3 respectively, while in intermediate groups it shows a regular increase up to the group with log carapace length = 1·17, followed by a regular decrease.

The relative growth of dentary margin/carapace length is also essentially as at Plymouth, the value of α being about 1·2. Dentary margin shows positive allometry, therefore, whilst frontal width shows negative allometry. Carapace width shows virtual isometry relative to carapace length (plotting from the data of Day (1935)), so that the growth of the two sections of the carapace width (Fig. 2) is, as it were, compensatory. The possibility that *b* for dentary margin/carapace length shows regular trends as in the case of frontal width has not been investigated; it might possibly be expected to show an annual increase, in view of the 'compensatory' tendency noted above.

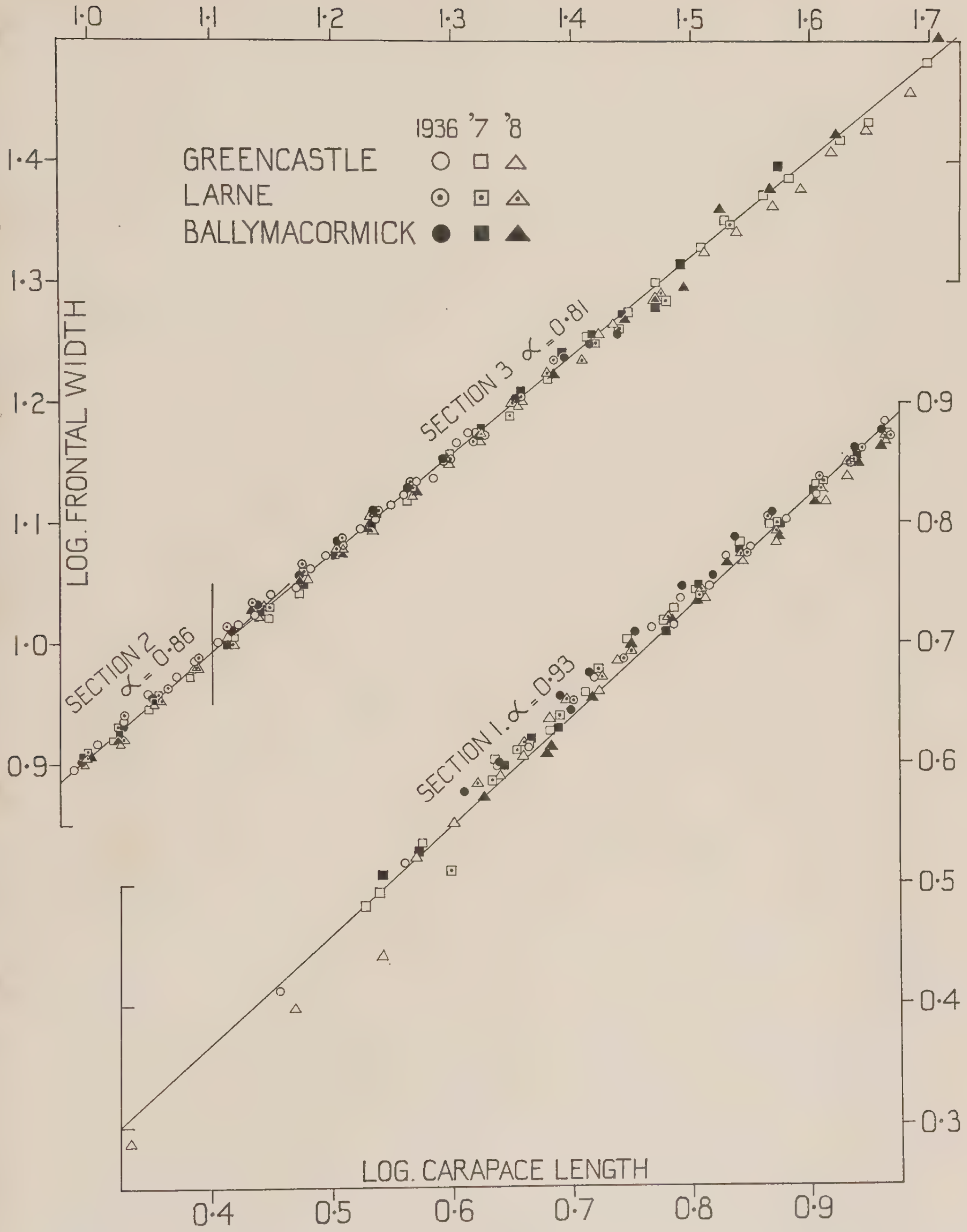

Fig. 6. Relative growth, frontal width/carapace length. Data (group points) for all nine Irish populations plotted on a single graph. Double logarithmic plotting. The curve is divided into three sections (1 in the lower graph and 2 and 3 above) containing approximately equal numbers of individuals. The slope of the line best fitting each section gave the values of α (0·93, 0·86, 0·81) used for the mathematical tests. It also shows: (1) variation in frontal width (vertical scatter of points) in each group is much greater than that in carapace length (horizontal scatter) due to chance variation in small samples only; (2) considerable differences between different groups in the extent of this variation in frontal width.

Notes on the Mathematical Tests

The assumption that α is the same for all nine populations, in any section, is fundamental to Teissier's test (p. 263); but, as indicated above (p. 267, Fig. 4), it is probable that α shows slight variations between the different populations, and it seems advisable to inquire how this might affect the validity of the tests. For an individual of average size (12 mm. carapace length) the substitution of $\alpha = 0.85$ for $\alpha = 0.80$ would produce a change in b of about 0·05, far greater than any difference recorded between the $\bar{b}$ values for any two populations. The range of values of α shown on Fig. 4 indicates that the error in applying a mean value of α to a particular curve might well be of this order. Moreover, every point on the curve will be subject to this error, since the method of calculating b amounts to drawing a line through each point parallel to the mean curve. Again, in comparing two population curves, one having a real α value above the mean and the other below the mean, the difference $\bar{b}_2 - \bar{b}_1$ will be doubly affected by this error. If the true $\bar{b}_2$ were not less than the true $\bar{b}_1$ the apparent difference would be too large, while if it were less the errors would tend to cancel each other out; recorded differences may therefore be too large or too small. What they actually represent is a difference in b and α combined, the effect of α being often considerable (cf. Reeve, 1940, Text-fig. 4). Where two curves diverge their difference will be exaggerated, and where they approach or cross the difference may be reduced to insignificance. It seems probable that much of the apparent irregularity in significance from one section to another in the detailed test (Table II) may be due to this cause; the considerable reduction in this irregularity in the general test is also explained—the actual irregularities are partly smoothed out and therefore not so grossly exaggerated by subsequent treatment.

There are, however, a number of reasons for not discarding the tests as valueless. The contrast revealed between the magnitude of annual differences and that of local differences is independent of the factor just considered. The consistent trends in annual, local and sectional differences in variability of $\bar{b}$ and in $\bar{b}$ itself must also be real. The differences clearly show the relation between any two curves, whether they diverge, approach or cross, by the sign and magnitude of the difference in successive sections, so that an accurate picture is obtained which could not be accepted with confidence from the graphs alone. Moreover, since both b and α do apparently vary between populations, a figure based on both may legitimately be considered the best estimate of the extent of the difference between populations. In any case b depends on the value of α in previous sections of the growth curve, and the two parameters are interdependent (cf. Lumer, 1939). The values of $\bar{b}$ shown in the tables give the mean position of that section of the curve in a vertical direction (i.e. direction of the ordinate)—the 'positional' value of Reeve (1940, p. 69). The differences between these $\bar{b}$ values give the extent to which the curves are separated in the vertical direction, throughout the section, and not merely at the beginning as would be the case with true values of $\bar{b}$. Thus the tests give valuable information about the differences between populations. Their only shortcoming is that they do not distinguish between differences due to b and those due to α. The Plymouth graphs strongly suggest that $\bar{b}$ is much the more variable, and there is support from the Belfast data. Teissier (1936) found that local races of *Homarus*, *Haliotis*, *Littorina*, etc., differ in b but not in α, and the greater variability of b has been demonstrated by many workers.

In further justification of the tests it should be borne in mind that the use of a value of α (i.e. the adopted value) other than the true value increases the *variance* of b whether it increases or decreases the value of b itself (Fig. 7). The variance involves the square of the standard deviation, and since a difference, $\bar{b}_2 - \bar{b}_1$, must exceed 2 × the square root of the sum of the two variances to be significant, there is every reason to believe that the test of significance is much more demanding than it would be with the true values of $\bar{b}$.

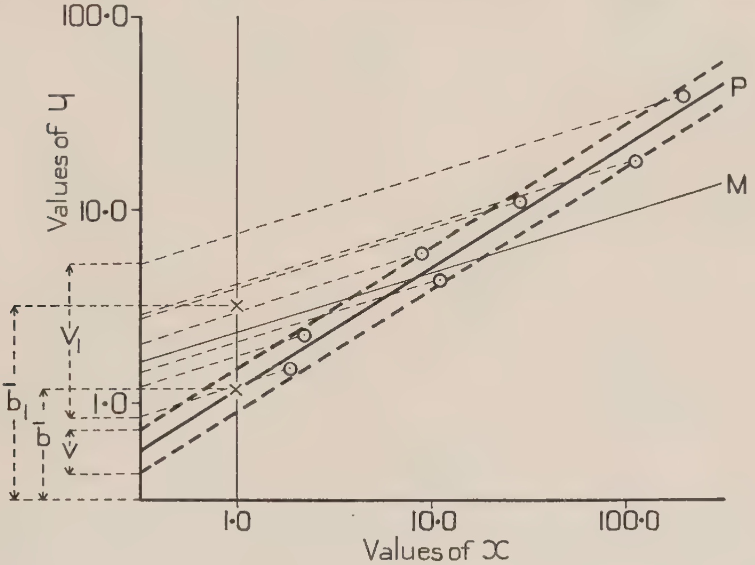

Fig. 7. Diagram to show the effect on $\bar{b}$ and on the variation of b produced in a population curve by the substitution of the mean value of α for the true value. M = mean curve for all populations. P = curve for population in question. b and v = true value of b and of its range of variation. $\bar{b}_1$ and v_1 = corresponding values obtained by using the mean value of α.

A possible source of error exists where the distribution of individuals is not uniform along the whole curve (or section). In the case of two curves not parallel to the mean curve, if one has many individuals near the beginning of the section and few in the second half, whereas the reverse holds for the other curve, then even if the two are virtually coincident (their α and b values both being the same) they will show very different apparent values of $\bar{b}$ (Fig. 8).

In the present work every effort was made to ensure even distribution of individuals, and in any case those body sizes which were poor in individuals (the smallest and the largest) corresponded fairly closely in all populations.

If α is not constant over any considerable range of body size, but changes continuously throughout each section, as seems very probable, the value of $\bar{b}$ obtained from the tests will be affected by this too. Here again the tests are of value; they still give an estimate of the *average* positional difference between two curves, in each section.

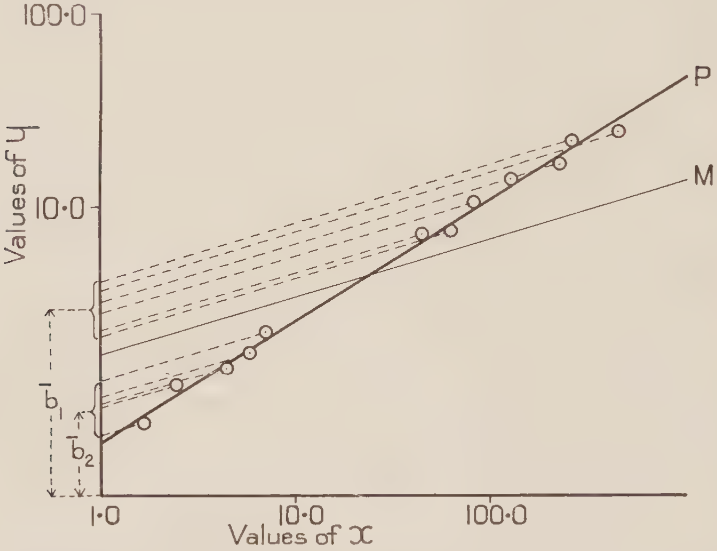

Fig. 8. Diagram to show how, when using the mean value of α instead of the true value, two coincident population curves give widely different values of b if there is not an even distribution of individuals along the curve. M = mean curve for all populations. P = coincident curves of the two populations in question. $\bar{b}_1$ and $\bar{b}_2$ = the mean values of b for the two populations.

RESULTS OF THE MATHEMATICAL TESTS

The value of b is given by y when $x=1$ in the allometry equation. Since the unit of x is a purely arbitrary quantity it may be taken as the value of x at the beginning of the particular section of the graph under consideration. In any case it will increase from section $1\rightarrow 2\rightarrow 3$, following the fall in α. The change is far greater than the differences between the populations within any section, but this does not affect the significance of the latter (Tables I–III), which depend only on the values of b within that section.

From the general test (p. 264) it is seen (Table I) that the value of $\bar{b}$ shows a regular annual decline, '36 > '37 > '38, and that there is a comparable regular sequence among the three localities, L, B, G, the widest frontal aperture being

TABLE I. THE GENERAL TEST

A. Yearly differences in $\bar{b}$, all localities combined

Year	Section	No. of individuals	$Sb/n=\bar{b}$	Variance of $b \times n$	Variance of $\bar{b} \times 10^6$	Comparison between years	Section	Difference $\bar{b}_2-\bar{b}_1$	Standard error of difference	Sign and significance of difference
1936	1	228	0·9798	0·3588	6·93330	1936–7	1	−0·002142	0·003027	−0
	2	458	1·1342	0·4558	2·17791		2	−0·011701	0·001913	+SSSSS
	3	320	1·2712	0·2360	2·31211		3	−0·008552	0·001921	+SSS
1937	1	421	0·9819	0·3939	2·22758	1937–8	1	−0·012239	0·002032	+SSSSS
	2	496	1·1225	0·3647	1·48542		2	−0·004127	0·001723	+S
	3	381	1·2627	0·1990	1·37464		3	−0·007568	0·001726	+SSS
1938	1	404	0·9697	0·3101	1·90477	1936–8	1	−0·010097	0·002973	+SS
	2	374	1·1183	0·2069	1·48313		2	−0·015828	0·001913	+SSSSSSS
	3	368	1·2551	0·2172	1·60852		3	−0·016120	0·001980	+SSSSSSS

B. Local differences in $\bar{b}$, all years combined

Locality	Section	No. of individuals	$Sb/n=\bar{b}$	Variance of $b \times n$	Variance of $\bar{b} \times 10^6$	Comparison between localities	Section	Difference $\bar{b}_2-\bar{b}_1$	Standard error of difference	Sign and significance of difference
Greencastle	1	425	0·9760	0·5562	3·08657	G–L	1	−0·002285	0·002345	−0
	2	535	1·1233	0·4993	1·74787		2	−0·003970	0·001957	−0
	3	423	1·2573	0·2921	1·63658		3	−0·009509	0·001726	−SSSS
Larne	1	327	0·9782	0·2574	2·41440	G–B	1	−0·000299	0·002492	−0
	2	347	1·1273	0·2504	2·08576		2	−0·003022	0·001849	−0
	3	387	1·2668	0·2004	1·34126		3	−0·007752	0·002095	−SS
Bally-macormick	1	301	0·9763	0·2817	3·11949	L–B	1	+0·001986	0·002316	+0
	2	446	1·1263	0·3318	1·67204		2	+0·000948	0·001939	+0
	3	259	1·2651	0·1840	2·75299		3	+0·001757	0·002022	+0

found in the most silty locality. However, whereas the annual differences are quite significant (except '36_1–'37_1), local differences are not (except G–L_3 and G–B_3). Mr Spooner's analysis of variance leads to the same conclusions: there are significant annual differences in all sections but local differences are only significant in section 3. The annual differences on the other hand decrease slightly, section 1→2→3.

The detailed tests (p. 264) reveal a number of irregularities which repay further investigation. The analysis of variance shows that the irregularities are greater than the variance within samples and are therefore significant. They are of the order of magnitude of the local differences but much smaller than the annual differences. The irregularities are greatest in section 1 and about half as great in 2 and 3. It seems possible that they are of the same nature as the local differences, that is, due to quite local causes.

The differences between '36 and '38 are consistently more significant than over either of the 1-year periods (Table II), showing that the annual differences are definitely progressive. This is most marked at B; at G, L there are often marked differences over a 1-year interval. In no 1-year interval is the difference significant in all three sections, but only at G '36–'37 is it insignificant in two of the three sections and usually it is far in excess of the limit of significance. In both G and B the order of the significance for 1, 2 in '36–'37 corresponds to that for 2, 3 in '37–'38 (−0 and +SSSSS for G, and +0 and +SSS for B), and this might be taken to indicate that the annual growth of *Carcinus* was approximately one section of the range of body size. However, the observations of Orton (1936) definitely disprove this. In any case the feature is not shown by L and is probably quite fortuitous.

L is anomalous in a number of respects. Its annual differences are smaller and less consistent than at G and B. In L_1 there is a significant increase in b from '36 to '38, instead of the usual decrease, and similarly in L_3 '37–'38.

Annual differences are greatest at Greencastle, the intermediate locality, and least at Larne.

The detailed tests bear out the general conclusion that local differences are less marked than annual differences: only 14/27 differences are significant as against 21/27 for the latter (Table II, B). Four of the nine comparisons show two sections insignificant (one only in the annual tests) and in seven of the nine the sign of the difference changes between sections 1 and 3, indicating that the two curves in question have crossed each other. In three sections ('36_2, '37_3, '38_2) none of the differences are significant, and only in '37_2 and '38_3 are all the three differences significant.

The value of the standard deviation of $\bar{b}$ has been calculated by Mr Spooner and shows interesting features. It decreases regularly '36 > '37 > '38 as does $\bar{b}$ itself, but the decrease is relatively greater; the decrease is shown in all sections (1 > 2 > 3) and in all localities (L least). Similarly there is a regular sequence in the localities G > B > L, involving all sections (1 most) and all years ('38 least). On the other hand, it is noteworthy that the locality sequence

TABLE II. THE DETAILED TEST. TEST FOR SIGNIFICANCE OF DIFFERENCES BETWEEN MEAN VALUES OF b FOR DIFFERENT POPULATIONS

Year, place and section			Difference $\bar{b}_2-\bar{b}_1$	Standard error of difference	Sign and significance of difference
		A. Yearly differences in each locality			
Greencastle		$'36_1-'37_1$	−0·008889	0·0050170	−0
		$'37_1-'38_1$	+0·024508	0·0033407	+SSSSSS
		$'36_1-'38_1$	+0·015619	0·0047223	+SS
		$'36_2-'37_2$	+0·021141	0·0030878	+SSSSSS
		$'37_2-'38_2$	−0·000728	0·0028660	−0
		$'36_2-'38_2$	+0·020413	0·0028940	+SSSSS
		$'36_3-'37_3$	+0·001239	0·0030720	+0
		$'37_3-'38_3$	+0·017874	0·0028627	+SSSSS
		$'36_3-'38_3$	+0·019113	0·0029460	+SSSSS
Larne		$'36_1-'37_1$	−0·012786	0·0057155	−S
		$'37_1-'38_1$	−0·003160	0·0032420	−0
		$'36_1-'38_1$	−0·015946	0·0055009	−S
		$'36_2-'37_2$	+0·005934	0·0030020	+0
		$'37_2-'38_2$	+0·009408	0·0030578	+SS
		$'36_2-'38_2$	+0·015342	0·0031353	+SSS
		$'36_3-'37_3$	+0·012393	0·0031780	+SSS
		$'37_3-'38_3$	−0·004677	0·0023130	−S
		$'36_3-'38_3$	+0·007716	0·0033029	+S
Ballymacormick		$'36_1-'37_1$	+0·006632	0·0041770	+0
		$'37_1-'38_1$	+0·017908	0·0039674	+SSS
		$'36_1-'38_1$	+0·024540	0·0045804	+SSSS
		$'36_2-'37_2$	+0·008349	0·0029770	+S
		$'37_2-'38_2$	+0·004014	0·0028480	+0
		$'36_2-'38_2$	+0·012363	0·0031220	+SSS
		$'36_3-'37_3$	+0·012938	0·0039740	+SS
		$'37_3-'38_3$	+0·009298	0·0037840	+S
		$'36_3-'38_3$	+0·022236	0·0036730	+SSSSS
		B. Local differences in each year			
1936	G–L	1	+0·013672	0·0067446	+S
		2	−0·000385	0·0044283	−0
		3	−0·009889	0·0036235	−S
	G–B	1	−0·007756	0·0054313	−0
		2	+0·003628	0·0031804	+0
		3	−0·011891	0·0035270	−SS
	L–B	1	−0·021428	0·0062193	−SS
		2	+0·004013	0·0044710	+0
		3	−0·002002	0·0039560	−0
1937	G–L	1	+0·009775	0·0036729	+S
		2	−0·015592	0·0029880	−SSSS
		3	+0·001265	0·0025690	+0
	G–B	1	+0·007765	0·0036222	+S
		2	−0·009164	0·0028770	−SS
		3	−0·000192	0·0036030	−0
	L–B	1	−0·002010	0·0036470	−0
		2	+0·006428	0·0028050	+S
		3	−0·001457	0·0032016	−0
1938	G–L	1	−0·017893	0·0028600	−SSSSS
		2	−0·005456	0·0029326	−0
		3	−0·021286	0·0026702	−SSSSSSS
	G–B	1	+0·001165	0·0036121	+0
		2	−0·004422	0·0028300	−0
		3	−0·008768	0·0031180	−S
	L–B	1	+0·019058	0·0036959	+SSSS
		2	+0·001034	0·0030968	+0
		3	+0·012518	0·0030692	+SSS

is the reverse of that due to $\bar{b}$ itself (p. 272). In the same way the regular decrease in the standard deviation through the three sections ($1 > 2 > 3$) is accompanied by a considerable increase in $\bar{b}$ itself.

The standard deviation is a measure of absolute variability; by dividing it by $\bar{b}$ the coefficient of variability is obtained. The annual differences in S.D. are so great ('38 only 75 % of the value for '36) that the much smaller decline in $\bar{b}$ itself (1½ % only) has little effect and the coefficient of variability also shows a marked annual decline. The difference between localities in the S.D. is much less marked than the annual differences, but the coefficient of variability shows more marked differences from the fact that the value of $\bar{b}$ itself shows the reverse order ($L > B > G$). This effect is even more marked in the sequence between sections where the inverse increase in $\bar{b}$, section $1 \rightarrow 2 \rightarrow 3$, is much greater.

TABLE III. THE DETAILED TEST. THE MEAN OF b AND ITS VARIANCE IN THE DIFFERENT POPULATIONS

Year, locality and section			No. of individuals	$\bar{b}$	$(Sb)^2/n$	$S(b^2)$	Variance of $b \times n$	Variance of $\bar{b} \times 10^6$
Greencastle	'36	1	119	0·9788	114·0038	114·2587	0·2549	18·1527
		2	218	1·1356	281·1373	281·3675	0·2302	4·8662
		3	118	1·2644	188·6444	188·7129	0·0685	4·9616
	'37	1	146	0·9877	142·4222	142·5707	0·1485	3·7015
		2	176	1·1145	218·6014	218·7452	0·1438	4·6688
		3	159	1·2632	253·6925	253·8050	0·1125	4·4781
	'38	1	160	0·9632	148·4291	148·5347	0·1056	4·1509
		2	141	1·1152	175·3583	175·4276	0·0693	3·5106
		3	146	1·2453	226·4045	226·4832	0·0787	3·7175
Larne	'36	1	29	0·9652	27·0117	27·0339	0·0222	27·3399
		2	51	1·1360	65·8153	65·8529	0·0376	14·7451
		3	104	1·2743	168·8738	168·9613	0·0875	8·1684
	'37	1	145	0·9779	138·6606	138·7959	0·1353	6·4799
		2	160	1·1301	204·3277	204·4361	0·1084	4·2610
		3	149	1·2619	237·2610	237·3037	0·0427	1·9363
	'38	1	153	0·9811	147·2579	147·3516	0·0937	4·0291
		2	136	1·1207	170·7989	170·8923	0·0934	5·0871
		3	134	1·2666	214·7989	215·0213	0·0608	3·3386
Ballymacormick	'36	1	80	0·9865	77·8605	77·9322	0·0717	11·3449
		2	189	1·1320	242·1835	242·3700	0·1865	5·2488
		3	98	1·2763	159·6314	159·7025	0·0711	7·4795
	'37	1	130	0·9799	124·8281	124·9305	0·1024	6·1061
		2	160	1·1236	202·0098	202·1016	0·0918	3·6085
		3	73	1·2633	116·5105	116·5542	0·0437	8·3143
	'38	1	91	0·9620	84·2150	84·2939	0·0709	9·6337
		2	97	1·1196	121·5951	121·6370	0·0419	4·4996
		3	88	1·2540	138·3914	138·4374	0·0460	6·0084

This last feature, a decrease in the variability of b with increase in body size, does not necessarily imply a corresponding decrease in the variability of y. It can be shown by calculation that a decrease in the variability of b is not inconsistent with a constant *relative* variability of y (i.e. with an increasing *absolute* variability of y, the S.D. increasing as $\sqrt{y}$). It would be interesting to know whether, in the present case, y does show constant relative variability—for a decreasing variability of b is consistent with a considerable range in the variability of y, both above and below the standard of constant relative variability.

It is clear that the above phenomenon results from the fact that the value of b depends not on that of x or y alone but on the ratio between them. A decreasing variability of b with increase in body size implies an increasing correlation between y and x (i.e. a more constant ratio y/x). This result seems to demand an explanation but cannot be considered further at this point.

Discussion

The results of the present work show a close similarity to those of Weldon (1894); the ratio frontal width/carapace length decreases with increase in body size in *Carcinus moenas*, and it may further show a progressive decrease, at corresponding body size, from year to year. The observations on individual growth (Fig. 3) are sufficient to show that the former is a feature of differential growth in the individual, as the population curves, derived from Weldon's Plymouth data, had suggested (Needham, 1935). In this connexion, however, it is only fair to point out that since the population curve is only the mean of very variable individual curves (p. 264) it is possible that its essential form might not be changed if some natural selection agency were systematically eliminating individuals with relatively wide frontal aperture, or with a relatively high value of α. At present, however, there is no evidence concerning the possible effect of such a factor on the curve of relative growth.

Previous criticisms (Cunningham, 1928, etc.) have probably been sufficient to prove that the amount of silt in the water could not be such a factor in the present case, but the deliberate choice of localities in the Belfast studies, according to the amount of silt, has enabled a clear demonstration of this. Frontal width is consistently greatest at Larne, where, according to Weldon's hypothesis, it should be smallest; it is smallest at Greencastle, where it should be intermediate, so that silt cannot be a determining factor.

The yearly decline in the ratio is undoubtedly the most interesting feature of the data. The previous suggestion (Needham, 1935) that it depended simply on an initial difference in the ratio at the outset of growth seems reasonable. Teissier (1934) has suggested similar differences in proportion in the Zoea larva to account for the same phenomenon in the closely allied genus *Portunus*. Since local differences are so insignificant in comparison with annual differences (p. 274) it seems probable that the cause of the annual differences in larval proportions must be sought in factors which affect all localities equally, for example, climatic, affecting the atmosphere, or hydrographic. The annual changes are clearly not continuous over long periods; at Plymouth b decreased by 0·007 in 5 years, so that in about 650 years it would approximate to zero! That the Belfast data overlap the Plymouth data of 40 years earlier also suggests that periods when b decreases are offset by others showing an annual increase. Whether the change follows a regular cyclic course, as in the case of fluctuations in animal numbers in many species (Elton, 1924), or is quite irregular cannot be decided without more data. There is strong evidence for the latter alternative however (Kemp, 1938). Hydrographic conditions in the Atlantic show considerable annual changes which appear to be quite irregular. It seems a priori probable that for marine animals hydrographic conditions may be the most important influence, far more so than direct climatic conditions.

It is of interest that there is a distinct sex difference in *b* in the same year (evidence from the data of Weldon), since local differences in any year are so small. However, this is presumably a genetic difference, quite independent of environmental factors.

The insignificance of local differences is indeed remarkable. The three localities differ in respects other than that of silt. Larne is very sheltered, with a very muddy substratum and with an admixture of fresh water, while B is a rocky coast exposed to the open sea; in all respects G is intermediate. Thus a number of local differences might be expected to combine to produce distinct local differences in relative proportions. The absence, in fact, of any such marked effects suggests that the animals may be relatively insensitive to most differences in local conditions. Considerable differences between local populations of terrestrial species are often recorded (Dice, 1940), but there it is probable that genetic differences may be at work; there is much less isolation among marine forms.

Such small local differences as there are (Table II B) do not follow the expected sequence; G is not intermediate and the order is L → B → G (p. 272). There is only one obvious factor which distinguishes G from the other two and might account for its position in the sequence: there is a local effluent from Belfast, and the general effect of a large city on the Lough may be considerable. The sequence G → B → L does, further, agree with their geographical latitude.

The anomalous features of the populations at L (p. 274) may well be due to its sheltered position, causing a general damping down of all environmental fluctuations. The constant outflow of fresh water might also have some effect, particularly on marine hydrographical changes. The tendency towards annual increases in *b* in some sections of the L data may well be due to these factors.

Local differences clearly exist (p. 274), but they are smaller and less consistent than annual differences. The fact that they tend to be more marked in section 3 than at smaller body sizes (p. 274) suggests a continuously operating type of influence, that is, a local environmental factor. At the same time it is just possible that initial differences are alone responsible: they would automatically increase with body size.

The probability that α changes continuously with body size means that the law of simple allometry (p. 267) does not strictly apply. This does not detract from the essential value of the law, however, and the assumption of a constant value of α over a section of the growth curve is justified by the facilities it provides for testing differences between populations, varieties and species. Reeve (1940) and others have already shown the value of the law, even as an approximation, in solving taxonomic problems. The idea of a regular change in proportions with increase in body size, and the very simple equation which effectively expresses the relation, have opened up possibilities which have not yet been fully exploited.

Another apparent criticism (p. 264), namely, that the curve of relative growth obtained by plotting the data of a large sample (population curve) is only a statistical mean and not an expression of individual growth (Davenport, 1934) does not detract from the value of the curve. The growth of an individual is as much a statistical mean (of the activity of its cells) as that of a population or of a species. The population curve is a population characteristic and may be used with the same confidence as any recognized character, morphological or otherwise. Again, the superiority of a mathematical estimate over purely descriptive morphological characters, as a permanent record, is undoubted.

No reason for the change in relative frontal width with increase in body size has been advanced. It may be a secondary effect of the contrasted (and apparently complementary) increase in relative size of the dentary margin (p. 268). The dentary margin corresponds roughly to the branchial region of the carapace and if, as seems probable, the surface area of the gills increases in simple proportion to the volume of the body as a whole, the dimension in question might be expected to increase relative to other dimensions of the body (roughly to the power $\sqrt{\tfrac{3}{2}} = 1{\cdot}23$ approx., which is roughly the value of α actually recorded for dentary margin/carapace length). If the relative decrease in frontal width is a compensatory result of this it must further be supposed that other considerations demand approximate isometry between total carapace width and carapace length.

ACKNOWLEDGEMENTS

Our thanks are due to Prof. T. Thomson Flynn for his continued interest in the work and for the facilities enjoyed in his Department, and to the Research Grants Committee of the Queen's University for their welcome financial assistance. We are extremely grateful to Dr Stanley Kemp for his interest in the MS. and for his valuable suggestion for its improvement, and to Mr E.C.R. Reeve and Mr G.M. Spooner for their indispensable help with the mathematical tests. We are indebted also to Dr Wishart of the Statistical Laboratory, Department of Agriculture, Cambridge, for advice and for the use of calculating machines.

It is hoped to deposit the original data, too bulky to be included here, with the library of the British Museum, Natural History.

Summary

1. Measurements of *Carcinus*, comparable to those taken by Weldon at Plymouth 40 years ago, have been made on material from three Irish localities, the observations in each area extending over three years. The three localities afford different environments, one having no silt, one a moderate amount and the other much silt.

2. The results support the view that the change in the ratio frontal width/carapace length with increase in body size is due to differential growth in the individual and is not caused, as Weldon supposed, by the continuous removal through natural selection of those crabs with a relatively wide frontal aperture. Measurement on the growth of individuals confirms this view.

3. A striking fact which emerged from Weldon's work was that the ratio mentioned above showed a successive diminution in each of the three years covered by the observations. A precisely similar diminution has been found at each of the Irish localities, and it is shown that the annual differences are mathematically significant.

4. The results disprove Weldon's hypothesis that the change in the ratio is correlated with the slow accumulation of silt in Plymouth Sound. The Irish locality with most silt has the widest frontal aperture and the intermediate locality the narrowest. It is also shown that the yearly trend towards a lower value for the ratio cannot be continuous, for the rate of change is too rapid to be maintained indefinitely, and the values obtained in the Irish localities overlap those at Plymouth 40 years earlier. Possible explanations of the changes are discussed.

5. The differences between populations from the three localities in any year are much less marked than the annual differences at one locality, and are not generally significant. Though small, however, they do show a consistent sequence among the three localities (but not corresponding to the order for siltiness).

6. The equation of simple allometry, $y = bx^{\alpha}$, applies to the data, at any rate as a useful approximation. The mean of b, and the variance of b, show consistent annual, and local differences in magnitude, and a regular change with increase in body size. The sequence for b itself may either correspond with that for its variance or run precisely counter to it.

7. The relation of individual growth to the mean curve of growth for a whole population is indicated and the value of the latter discussed.

8. A possible explanation of the differential growth of the dimension frontal width is advanced.

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SACCOGLOSSUS HORSTI SP.N., AN ENTEROPNEUST OCCURRING IN THE SOLENT

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(Text-figs. 1–8)

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INTRODUCTION

The species described in this paper was found at Lymington by one of the authors (C.B.G.) in the summer of 1940. It differs from the three species of this genus that have been described from the British coasts and from *Saccoglossus kowalevskyi* Agassiz, the American species, which Caullery & Mesnil (1916) claimed to have found on the French coast of the English Channel at St Martin, near Cap de la Hague. Its characters, though distinctive, are such as might lead to confusion with *S. kowalevskyi*. It seems possible, therefore, that the record from the coast of France may refer to this and not to the American species. Since St Martin is directly opposite Lymington and only some 75 miles distant, this explanation appears more probable than that the French record really refers to the American species, especially when it is remembered that, so far as is known (van der Horst, 1927–39), all other species of *Saccoglossus* have very restricted distributions. Doubtless the local character of the species of this genus, and indeed of the other members of the Harrimanidae, is associated with their direct development and it contrasts sharply with the wide distribution of several members of the Ptychoderidae, in which a free-swimming stage in development, the *Tornaria* larva, provides for dispersal.

The specific name is chosen in recognition of Prof C. J. van der Horst's many contributions to the subject and in particular his exhaustive account of the Enteropneusta in Bronn's *Klassen und Ordnungen des Tier-Reichs*.

ENVIRONMENT

S. horsti occurs on the Hampshire coast, near the mouth of the Lymington River which runs into the western end of the Solent. The species extends about half a mile both east and west of the mouth of the river, but does not go up the estuary, where the mud soon becomes foul and black below the surface. It occurs in the deep grey mud (see Appendix for analysis), which is of the most glutinous type, from just above low-water mark spring tides to a short way above low-water mark neap tides. The animals live about 4–8 in. below the surface in rather diffuse burrows and no 'casts' could be distinguished at the surface. They occur also higher up the shore at about half-tide mark in the chunks of dead rhizomes of *Spartina Townshendii* Groves which are scattered on the surface of the soft mud, having broken away from the edge of the *Spartina* flats which fringe the Solent. Their burrows in this situation are in the under surfaces of those chunks of rhizomes which are not deeply embedded in the mud. Such pieces lie in pools of water and so the animals below them are never completely uncovered at low tide. The proboscis was found protruding from the mouth of the burrow when the chunk was lifted, while the tail went vertically upwards into the mass of dead roots. They do not occur in those masses so deeply embedded in the mud as to have their under surfaces foul and black, nor in the mud itself at this level.

The environment of *S. horsti* thus is strikingly different from that of *S. cambrensis*, Brambell & Cole (1939*a*), which occurs in Wales in relatively clean sand and fine shell gravel and is absent from adjoining stretches of mud flats. Moreover at Hurst Castle, 4 miles to the west of the Lymington River, there are flats uncovered at low-water spring tides, grading from quite clean to distinctly muddy sand, in which *S. horsti* could not be found. It was observed in the laboratory that when living specimens of *S. horsti* were put in a dish over mud they soon burrowed down, while over sand they lay on the surface and made no attempt to burrow but formed loose tubes of sand grains and mucus in which, however, they seemed to live quite happily. It appears, therefore, that *S. horsti* is essentially a mud-living species whereas *S. cambrensis* is sand-living.

S. horsti occurs in association with the amphipod, *Corophium volutator* (Pallas). It is very common in the mud and more numerous than all the annelids put together; in a good patch there may be half a dozen specimens in a trowelfull of mud.

MATERIAL AND TECHNIQUE

S. horsti is fragile and liable to fragment when handled, yet it is more robust and fragments less readily than *S. cambrensis*. Moreover, it is much more amenable to narcotization and fixation. Although each of the authors is well acquainted with either one or other of these two species neither has had an opportunity of examining both in the living condition. Therefore these conclusions are based on comparison of preserved specimens and of notes on the preservation. The specimens show in a striking manner that much more perfect examples can be obtained of *S. horsti* than of *S. cambrensis* and that they can be narcotized and preserved in a much more expanded state. Commercial specimens of *S. kowalevskyi* indicate that probably this species is still more readily preserved intact and in a flaccid condition. It may be significant that these differences are associated with differences in the epidermis and in the development of the musculature, which in turn may be related to the nature of the substratum in which the animal lives.

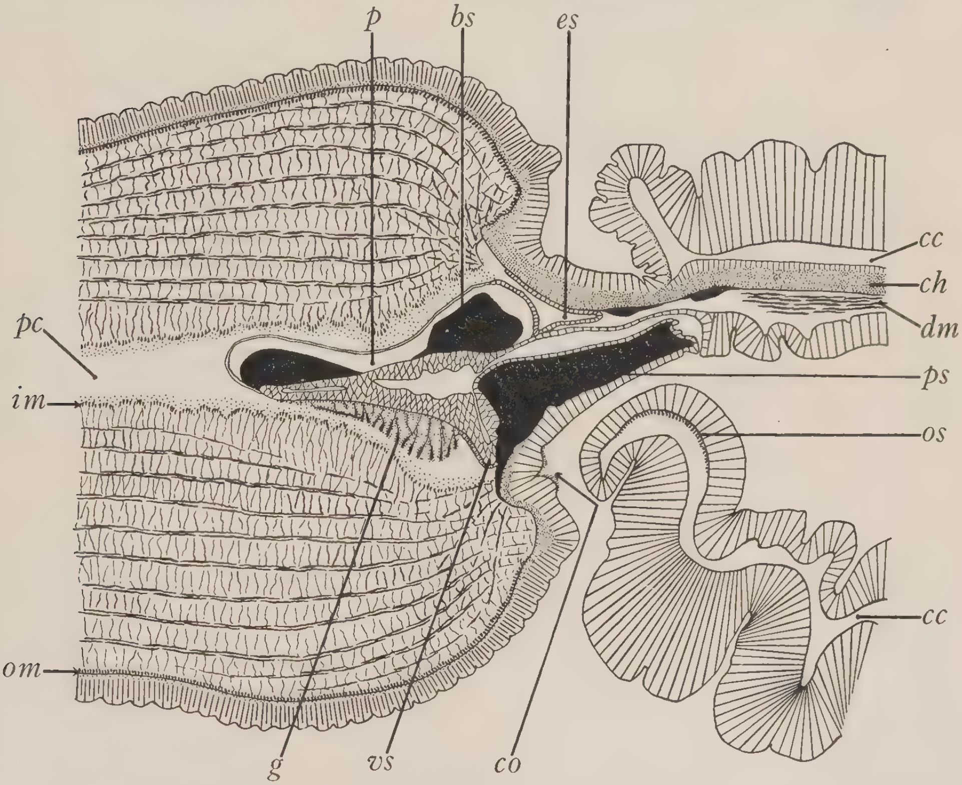

Fig. 1. Longitudinal sagittal section of the base of the proboscis. ×37. *bs.* blood sinus; *cc.* coelomic cavity of collar; *ch.* nerve cord of collar; *co.* preoral ciliary organ; *dm.* longitudinal muscle fibres of trunk in perihaemal cavity; *es.* end-sac; *g.* glomerulus; *im.* inner circular muscle fibres of proboscis; *om.* outer circular muscle fibres of proboscis; *os.* oral sphincter; *p.* pericardium; *pc.* coelomic cavity of proboscis; *ps.* body of proboscis skeleton; *vs.* ventral diverticulum of stomochord.

Narcotization was effected readily both by the menthol and by the gradual alcoholization methods, provided the animals were treated soon after collection. Specimens kept in the laboratory for some hours, though apparently healthy, responded to narcotization by copious secretion of mucus and by autolysis. Specimens, both narcotized and without narcotization, were fixed either in formalin or in Bouin's fluid. Thus both expanded and contracted examples were available for comparison. Both fixatives gave excellent results. Serial sections were cut at a thickness of 10μ and were stained with Ehrlich's haematoxylin and either eosin or Tischutkin's orange G-erythrosin. The latter stain was suggested to us by Dr N.B. Eales and has proved to be especially useful for the definition it imparts to epidermal basement membranes and blood sinuses.

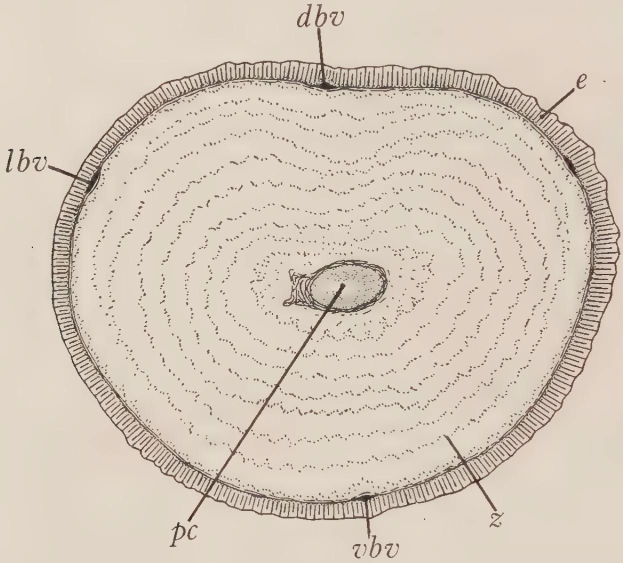

Fig. 2. Transverse section of contracted proboscis. ×24. *dbv*. dorsal blood vessel; *e*. epidermis; *lbv*. lateral blood vessel; *pc*. proboscis coelom; *vbv*. ventral blood vessel; *z*. concentric zone of longitudinal muscle fibres.

SPECIFIC CHARACTERS

Externals

The total length of one complete specimen, but not a large one, was 20·5 cm. when narcotized. Another specimen, which was a very large one but was incomplete posteriorly, was 31·5 cm. long and it is estimated that it must have exceeded 35 cm. in length when complete. The partly contracted proboscis of large living specimens measured 1·3–1·8 cm. long and 4–5 mm. in diameter at the thickest part near the base. The proboscis of narcotized specimens, in which it appeared to be fully extended, was 2·8–3·2 cm. long. The collar in large narcotized specimens was 4·5–5·5 mm. long in the dorsal middle line and 3·0–4·0 mm. long ventrally; it was 3·5–4·0 mm. in diameter anteriorly and 4·0–4·5 mm. in diameter posteriorly. Thus this species is slightly larger than *S. cambrensis*.

There is a very distinct dorsal groove reaching from the base to the tip of the proboscis. It is sufficiently marked posteriorly to render the base of the proboscis almost heart-shaped when viewed from above. There is also quite a distinct ventral groove reaching almost to the tip of the proboscis in the living animal. It is not apparent in material fixed with the proboscis contracted, but is distinguishable in specimens which were narcotized and in which the proboscis was preserved in an expanded condition. A preoral ciliary organ, similar to that of *S. cambrensis* (Brambell & Cole, 1939*b*), but less apparent in the living animal, since its coloration is not distinctive, is present on the base of the proboscis.

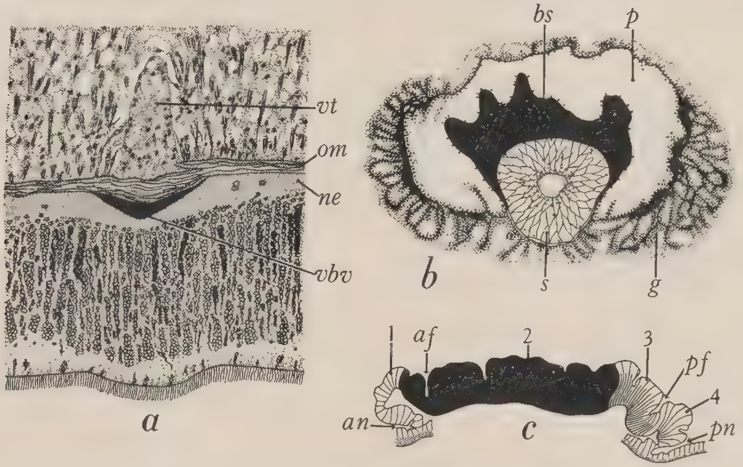

Fig. 3. *a*. Transverse section of ventral region of contracted proboscis shown in Fig. 2. ×150. *b*. Transverse section of the proboscis organs. ×60. *c*. Dorsal longitudinal sagittal section of the epidermis of the collar. The epidermal zones are numbered 1 to 4 from anterior to posterior. ×17. *af*. anterior furrow; *an*. anterior neuropore; *ne*. nerve fibre layer of epidermis; *pf*. posterior furrow; *pn*. posterior neuropore; *s*. stomochord; *vt*. ventral longitudinal tract overlying the ventral blood vessel; other guide letters as in previous figures.

The anterior border of the collar is slightly thickened and the posterior quarter is more definitely so. Posteriorly the margin of the collar overhangs the first two or three gill pores, forming a slight operculum.

The ventral muscle bands of the trunk project in the branchial and genital regions as a conspicuous rounded keel (Figs. 7, 8*b*). When the animal contracts this keel shortens more than the dorsal pharyngeal region. Consequently the muscular keel becomes more or less straight while the dorsal region coils sinuously around it. The gonads begin about 1 mm. behind the collar and extend a considerable distance behind the branchial region, overlapping the beginning of the hepatic region. They attain their greatest development at the anterior extremity of the oesophageal region, where they form rounded projecting ridges on each side of the body (Fig. 7). The genital ridges and the muscle bands, though projecting distinctly, are never so pronounced as those of *S. kowalevskyi* (Spengel, 1893). The number of branchial pores varies from specimen to specimen, as is the rule in allied species. The number of gill slits in three adult specimens in which they were counted were 104 pairs, 115 pairs and, in one large specimen in which the series was not quite complete, 137 pairs. Thus they are more numerous than in any other known species of the genus.

The animal has two clearly distinguishable colour forms occurring in approximately equal numbers and intermixed in the same environment. The paler form has a yellowish white proboscis with a brown base and a rust-brown collar tinged with orange with a white ring round the posterior margin. The darker form has a salmon-pink proboscis with a brown base and with a brick-red collar with a white ring round its posterior margin. The colour of the trunk is the same in either variety. The branchial region is a rather transparent grey tinged with salmon-pink and there is no deep red patch such as is found in *S. cambrensis* just behind the collar on each side of the body. The pink gonads show through the body wall; in some specimens their colour is pronounced. The trunk immediately behind the branchial region is a rich dark brown and thereafter it gradually pales towards the posterior extremity, which is fawn. The whole of the trunk is sprinkled with numerous small raised spots that are paler in colour than their background. No carmine spots, like those in *S. cambrensis*, were observed. Throughout the trunk the dorsal nerve cord is sharply defined with a pale margin on each side.

Proboscis

The nerve-fibre layer of the epidermis is well developed throughout the proboscis, but is thickened over the basal region (Fig. 1), where the epidermis is less glandular and more strongly ciliated than elsewhere. A preoral ciliary organ, so similar to that described in detail in *S. cambrensis* that it does not merit separate description (Brambell & Cole, 1939*b*), is present. The dorsal groove of the proboscis is more pronounced than in *S. cambrensis* or *S. kowalevskyi* and the nerve-fibre layer beneath it is thickened, forming a longitudinal tract lying between the groove and the dorsal subneural blood vessel. The ventral longitudinal groove is apparent in transverse sections of the extended proboscis, but is not accompanied by any thickening of the nerve-fibre layer, which tends rather to be reduced in thickness where it overlies the ventral longitudinal blood vessel (Fig. 3*a*). The circular muscle layer is fairly well developed and is as thick as, or slightly thicker than, the nerve-fibre layer. It is not thickened in the form of a sphincter at the end of the proboscis. The longitudinal muscles are arranged in nine or more concentric rings (Fig. 2) which are apparent even at the centre, though more clearly seen at the periphery. This complete concentric arrangement of the longitudinal musculature resembles that of *S. kowalevskyi*, although it is not so clearly defined as in that species, rather than that of *S. cambrensis*, and it distinguishes the species from the two remaining British species, *S. ruber*, Tattersall (1905) and *S. serpentinus*, Assheton (1908). The cavity of the proboscis is narrow, having a diameter at the narrowest part in the anterior half of the contracted proboscis of about one-tenth that of the whole organ. It is nearly filled with rounded coelomic cells, with very vacuolated cytoplasm and relatively small nuclei, which are either attached to its wall or floating freely in it. A thin layer of circular muscle fibres bounds the cavity (Figs. 1 and 2), which is not enlarged at the anterior end. Within the outer circular muscle layer and overlying the ventral blood vessel the stromal tissue is slightly differentiated to form a longitudinal tract, conical in cross-section (Fig. 3*a*), which can be distinguished only in well-preserved material. This tract appears to be free from longitudinal muscle fibres. Posteriorly it is continuous with the ventral extremity of the ventral septum through which the ventral blood vessel runs. Its function is obscure, but its structure and position are suggestive of some sort of neuromotor organ. The ventral septum is very short (Figs. 4*a* and *b*), extending only about 100μ in front of the caudal ends of the shallow ventral coelomic sacs. The left dorsal coelomic sac is connected to the wide median end sac, from which the proboscis pore opens to the exterior on the left side of the neck of the proboscis (Fig. 4). The stomochord is straight with a wide and continuous lumen throughout (Fig. 1). It is provided with a single ventral diverticulum. The walls of that region which lies above the proboscis skeleton

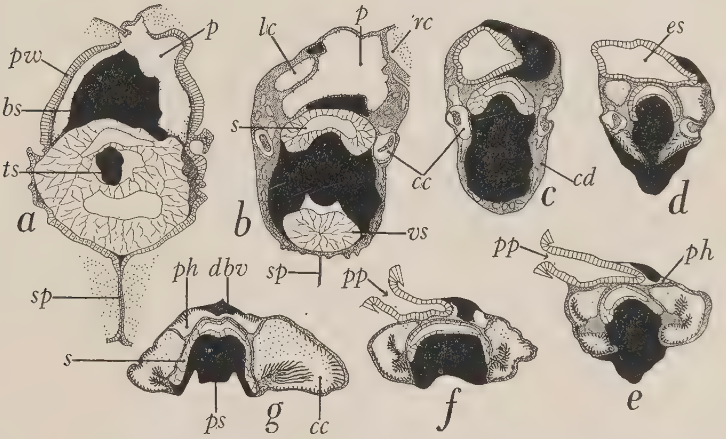

Fig. 4. Series of seven transverse sections at intervals of 100μ through the neck of the proboscis, showing the relations of the coelomic cavities, blood vessels, etc., and the shape of the body of the proboscis skeleton. Blood vessels and skeleton shown in solid black. The crura of the skeleton diverge within 50μ of the last section depicted in *g*. × 45. *cc.* extension of coelomic cavity of collar; *cd.* chondroid tissues; *lc.* left dorsal pouch of proboscis coelom; *ph.* perihaemal cavity; *pp.* proboscis pore; *pw.* pericardial wall, very thick in this region; *rc.* right dorsal pouch of proboscis coelom; *sp.* ventral septum of proboscis; *ts.* tip of proboscis skeleton; other guide letters as in previous figures.

are very thin (Fig. 4). Thus the stomochord is similar in shape to that of *S. kowalevskyi* and differs from that of *S. cambrensis*, in the absence of an S-shaped bend, in being shorter and stouter and in having thinner walls posteriorly. This character alone readily distinguishes the species from *S. cambrensis*. The large pericardium extends more than half-way round the stomochord (Fig. 3*b*). The glomerulus is continued around the stomochord ventrally and the right and left halves extend considerably more than half-way towards the mid-dorsal line of the pericardium, though there is no dorsal glomerulus. The form of the body of the proboscis skeleton (Figs. 1 and 4) is similar to that of *S. cambrensis* and the crura embrace between one-half and two-thirds of the circumference of the buccal cavity, though they only extend backwards between one-quarter and one-third of the length of the collar from its anterior margin. No hard concretion, like that described in *S. cambrensis*, was found in the core of the proboscis skeleton of any specimen.

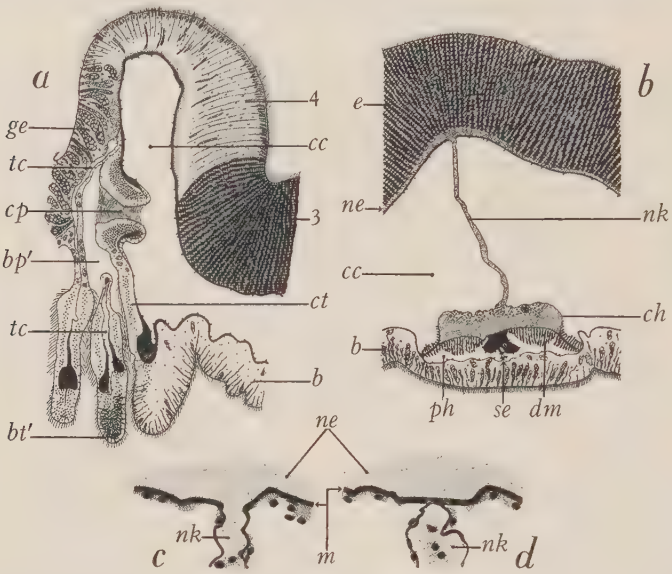

Fig. 5. *a*. Longitudinal section through the region of the collar pore. ×60. *b*. Transverse section through the dorsal region of the collar. ×45. *c* and *d*. Two transverse sections 30μ apart through the junction of the neural keel with the epidermis, showing an interruption in the epidermal basement membrane (*m*) in *c* and its continuity in *d*. ×325. *b*. epithelium of buccal cavity; *bp′*. first branchial pouch; *bt′*. first tongue; *cp*. collar pore; *ct*. collar-trunk septum; *ge*. branchial epidermis; *nk*. neural keel; *se*. septum between perihaemal cavities; *tc*. trunk coelom; other guide letters as in previous figures.

Collar

There are four epidermal zones in the collar (Fig. 3*c*). The first forms the anterior margin of the collar and is composed mainly of ciliated cells; the second, in which basophil glandular cells are predominant and the epidermis is thickest, extends over the anterior two-thirds of the surface; the third does not stain so densely basophil as the second, but glandular elements are numerous in it and the epidermis is almost as thick; the fourth, which is composed mainly of ciliated cells with relatively few glandular elements, forms the posterior margin. The first, second and fourth zones evidently correspond to the first, second and fifth zones of the collar respectively in *S. cambrensis*, while the third zone probably represents both the third and fourth zones of the Welsh species. The number of epidermal folds in the collar varies according to the state of contraction, but one deep fold in the second zone near the anterior margin and another between the third and fourth zones appear to be constant and are responsible for the appearance of rims around the anterior and posterior margins.

The right and left coelomic cavities of the collar are separate from each other. The ventral mesentery is complete. Dorsally the coelomic cavities are separated by the nerve cord, joined throughout its length to the epidermis by a pronounced keel, by the perihaemal cavities, the stomochord and the proboscis skeleton. Anterior prolongations of the collar cavities extend throughout the neck of the proboscis, reaching almost to the level of the anterior extremity of the proboscis skeleton (Fig. 4). The epithelium of the collar pores is thick and the nuclei of the cells are arranged in many rows (Fig. 5*a*). The two perihaemal cavities in the collar are completely separated from each other posteriorly by the septum, in which the dorsal subneural blood vessel runs (Fig. 5*b*). They join at the level of the posterior extremities of the crura of the proboscis skeleton and the dorsal subneural blood vessel in front of this point runs in the dorsal wall of the single perihaemal cavity, between it and the nerve cord (Fig. 4). This median perihaemal cavity extends into the neck of the proboscis as far as the proboscis pore (Fig. 4*e*). The anterior extensions of the dorsal longitudinal muscles of the trunk extend throughout the length of the perihaemal cavities. The collar-trunk septum is deflected forwards on each side of the buccal cavity ventro-laterally, as in *S. cambrensis* and *S. kowalevskyi*, forming triangular diverticulae of the trunk cavities which taper anteriorly and end a short distance behind the extremities of the crura of the proboscis skeleton. Prolongations of the ventral longitudinal muscle bands of the trunk extend throughout the length of these cavities, the fibres being attached anteriorly to the posterior surface of the septum. These muscles, together with the longitudinal muscle fibres of the collar which are attached to the anterior wall of the septum and to the crura of the proboscis skeleton, form presumably the retractor mechanism of the collar and of the neck of the proboscis.

The nerve cord is solid throughout its length, the anterior and posterior neuropores, if the shallow depressions which represent them justify the name, ending blindly. The neural crest or keel, connecting the nerve cord with the epidermis throughout its length, is well developed (Fig. 5*b*). The tissues of this keel are in direct continuity with the nerve-fibre layer of the epidermis at several separate points throughout the length of the collar, but in the intervening regions they are separated by the basement membrane of the epidermis (Fig. 5*c* and *d*). The points of continuity are in the nature of perforations in the basement membrane, perhaps 10μ in diameter, and are not associated with any local thickening of the keel. This is similar to the condition in *S. kowalevskyi* and is intermediate between that in some species, such as *S. cambrensis*, in which there is no continuity between the keel and epidermis, the continuous basement membrane intervening, and that in others, such as *S. inhacensis*, Kapelus (1936), and *S. pusillus* (Ritter) (van der Horst, 1930), in which the tissues of the keel are in uninterrupted continuity with the epidermis.

Circular muscle fibres in the collar cavities around the mouth form an oral sphincter (Fig. 1). The buccal cavity has no dorsal diverticulum, such as is found in *S. gurneyi* (Robinson, 1927) and *S. otagoensis* (Benham) (van der Horst, 1930).

Trunk

The epidermis of the branchial region surrounding the gill pores is characteristic (Fig. 8*a*). It is strongly ciliated and is packed with long narrow glandular elements, filled with homogeneous secretion which stains intensely with eosin. Consequently this epithelium can be distinguished in sections, even with the naked eye, by its eosinophility. Scattered among the eosinophil elements are a few basophil goblet cells, oval or pear-shaped in form with vacuolated cytoplasm. These are more numerous where the branchial epidermis joins that of the collar. The branchial epidermis thus differs widely from that of *S. cambrensis*, in which the dominant glandular elements are mucous-secreting goblet cells which appear vacuolated and partly disorganized with little affinity for stains, since large numbers of them discharge their secretions before fixation. The epidermis of the trunk behind the branchial region is thinner and is composed mainly of ciliated cells with relatively few glandular elements, except for numerous scattered patches of thicker, more glandular, epithelium.

The dorsal nerve cord is clearly defined in the branchial region, but there is no definite dorsal groove in any part of the trunk. The ventral nerve cord, which is much the larger, lies above a shallow ventral groove that extends throughout the branchial region, and gradually fades out in the oesophageal region. Otherwise the epidermal nerve-fibre layer is fairly well developed throughout the branchial region, but is very thin behind it.

The ventral longitudinal muscles of the trunk are well developed and the dorsal longitudinal muscles form two much smaller bundles extending throughout the branchial region (Fig. 6).

The dorsal blood vessel runs in the dorsal mesentery of the gut, which is continuous throughout the trunk. Anteriorly the epidermal basal membrane of the ventral nerve cord is in contact with the wall of the pharynx in the mid-ventral line, there being no ventral mesentery in this region in consequence (Fig. 6). Throughout the remainder of the trunk there is a continuous ventral mesentery, containing the ventral blood vessel (Figs. 7, 8*b*, 8*c* and 8*d*). Thus the two trunk cavities do not appear to communicate at any point.

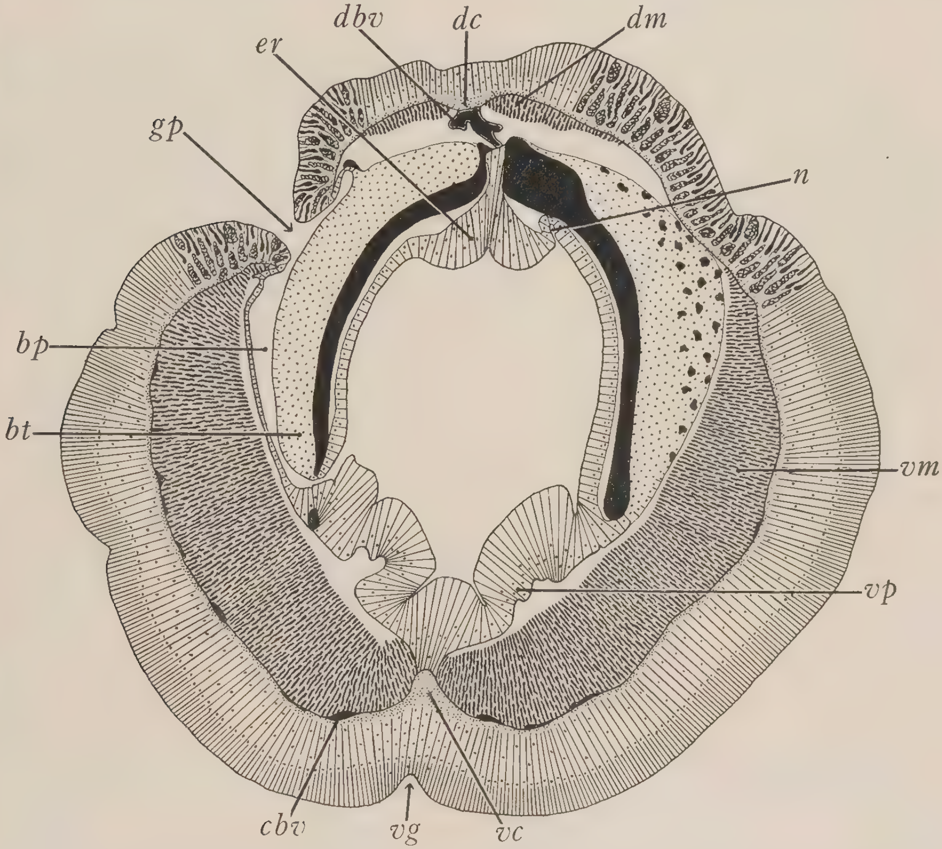

Fig. 6. Transverse section through the anterior part of the branchial region, passing through a branchial pore and tongue on the left and a septum on the right. ×46. *bp*. branchial pouch; *bt*. tongue; *cbv*. circular blood vessel; *dbv*. dorsal blood vessel; *dc*. dorsal nerve cord; *dm*. dorsal longitudinal muscle band of trunk; *er*. epibranchial ridge; *gp*. branchial pore; *n*. niche or recess between septum and epibranchial ridge; *vc*. ventral nerve cord; *vg*. ventral groove; *vm*. ventral longitudinal muscle band of trunk; *vp*. ventral non-branchial region of pharynx. Skeletal rods and blood vessels shown in solid black.

The gill slits embrace the dorsal two-thirds of the pharynx (Fig. 6). At the posterior extremity of the series the last few gill slits decrease progressively in size but tongues are present even in the last and smallest. Concretions such as those described in *S. cambrensis* are not found in the skeletal bars of this species. The tongues are broader and project farther into the lumen of the pharynx than do the septa (Fig. 8*a*). The ciliated epithelium covering the inner surface of the tongues and septa is similar, except that that on the tongues is thicker and contains many basophil glandular elements while that on the septa contains few. A thick epithelium, with long cilia and regularly arranged nuclei but devoid of glandular elements, covers the sides of both the tongues and septa. The epithelium lining the gill chambers and covering the outer surfaces of the tongues is not ciliated and the nuclei are not regularly arranged. Numerous large stellate pigment cells are scattered throughout this epithelium, except where it covers the tongues. The epibranchial ridge is grooved longitudinally in the mid-dorsal line and hence appears bilobed in transverse sections (Fig. 6). It differs in this respect from the epibranchial ridges in both *S. cambrensis* and *S. kowalevskyi*. The epithelium on the inner surfaces of the septa at their dorsal extremities, where they join the epibranchial ridge, is reduced in thickness, thus forming a niche

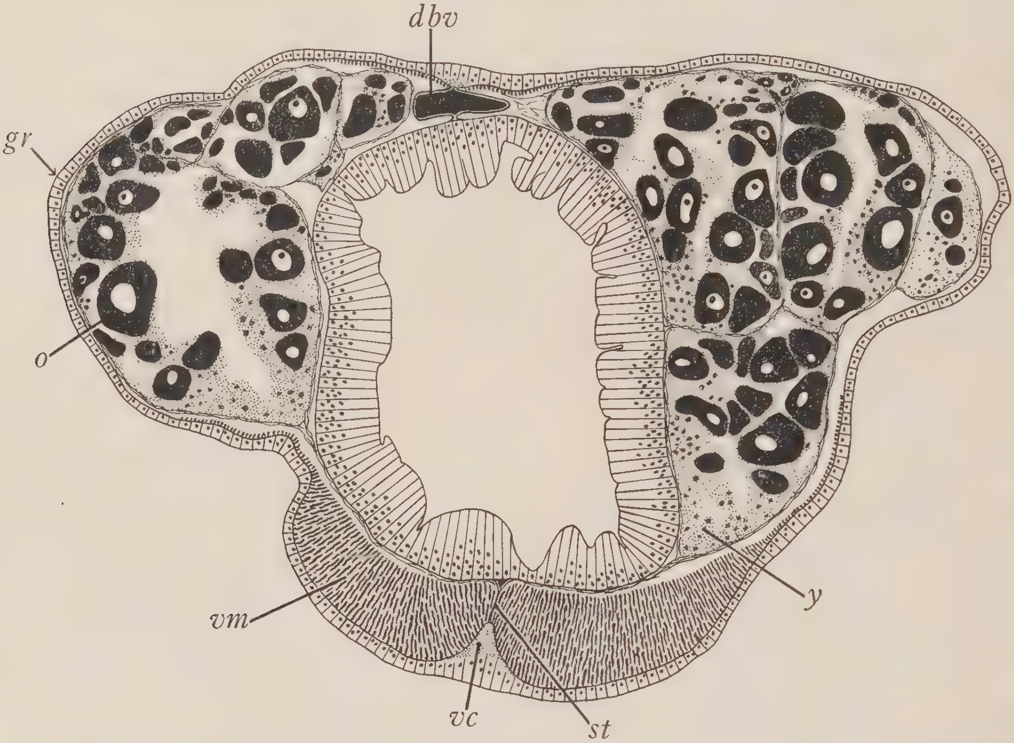

Fig. 7. Transverse section through the first region of the oesophagus showing the maximum development of the genital ridges. × 46. *gr*. genital ridge; *o*. oocyte in ovary; *st*. ventral septum of trunk; *y*. yolk-cells in ovary; other guide letters as in previous figures.

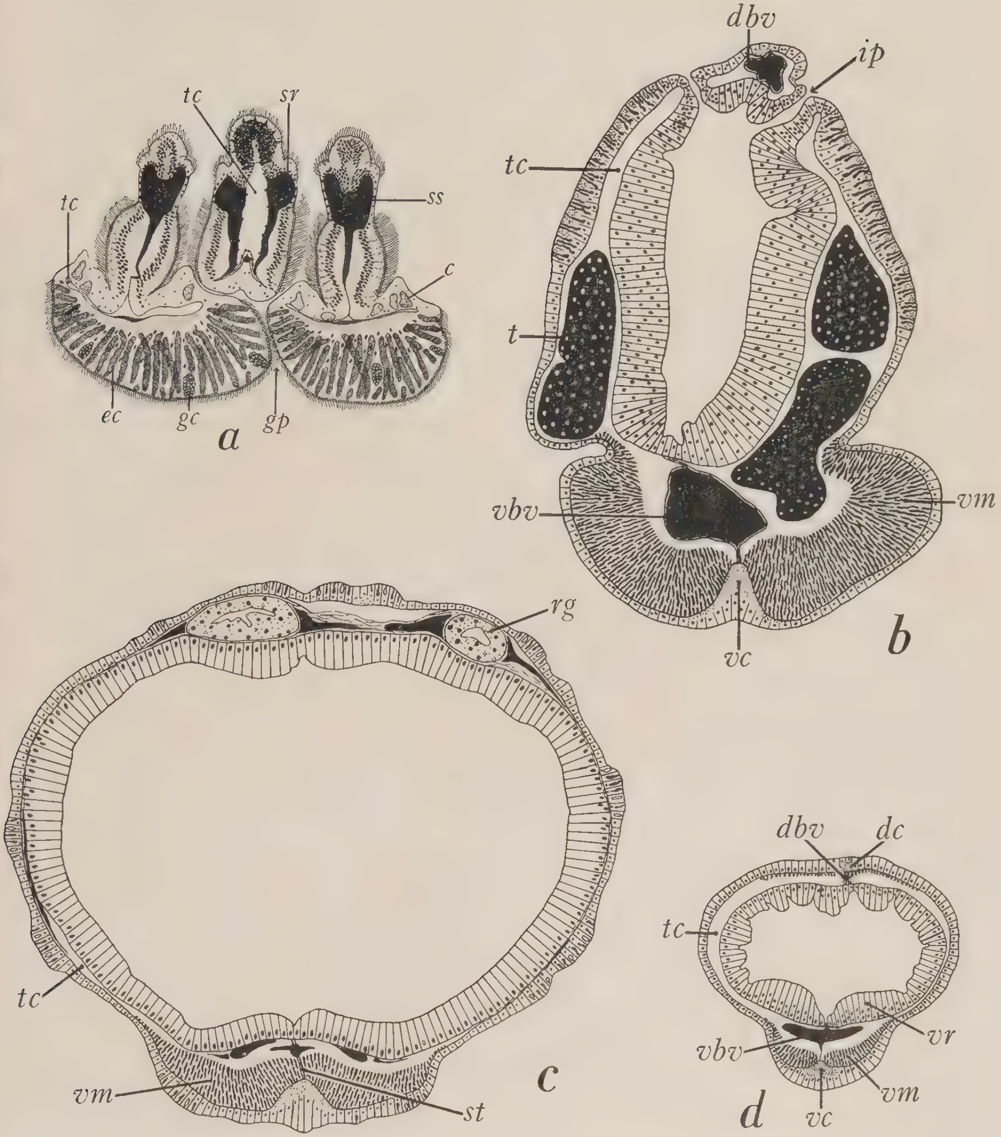

Fig. 8. *a*. Longitudinal horizontal section through two branchial septa, a tongue and a branchial pore. The character of the branchial epidermis is shown. ×127. *b*. Transverse section through the beginning of the second region of the oesophagus showing intestinal pores. ×46. *c*. Transverse section through the hepatic region. ×46. *d*. Transverse section through the intestinal region. ×46. *c*. pigment cell; *ec*. eosinophil glandular cell of epidermis; *gc*. goblet-cell; *ip*. intestinal pore; *rg*. rudimentary gonad; *sr*. skeletal rod in tongue; *ss*. skeletal rod in septum; *t*. testis; *vr*. ventrolateral ridge of intestinal epithelium; other guide letters as in previous figures.

or recess on each. No such recesses are found on the dorsal ends of the tongues. The epithelium of both the epibranchial ridge and the ventral non-branchial part of the pharynx resembles that of the buccal cavity. It is thick, strongly ciliated and contains a number of basophil glandular elements.

The oesophagus is relatively short, though, owing to contraction, its length could not be measured with precision. It is divided into three distinct regions. The epithelium lining the first (Fig. 7) resembles that of the ventral non-branchial part of the pharynx, but the glandular elements are rather more numerous and are regularly arranged, the oval bodies of the cells occupying the central zone of the epithelium and not extending into the basal zone, while their narrow necks traverse the peripheral zone to reach the surface. The second region has much thicker walls. A transverse section through the middle of this region reveals a cruciform lumen. The thickest epithelium lines the dorsal arm of the lumen. It is full of basophil glandular elements. The epithelium of the horizontal arms of the lumen is much thinner and is sharply differentiated from the rest by its lack of chromophility. The epithelium of the ventral arm is very thick, though not so thick as that of the dorsal arm, and it stains still more densely with haematoxylin. The horizontal arms of the lumen constitute two lateral longitudinal grooves in the wall which begin dorsally and slope backwards and downwards. The intestinal pores open into these grooves at their anterior and dorsal extremities (Fig. 8*b*). The epithelium lining these pores is similar to that lining the grooves into which they open internally. The number of pores varies from 4 to 8 in different specimens and indeed on the two sides of the same specimen. The numbers observed were 4+5, 5+5, 5+7 and 7+8. No rudimentary pores such as those described in *S. cambrensis* were observed. The third region of the oesophagus is lined by thinner epithelium, less crowded with glandular elements and distinguished by the oval nuclei being confined to the basal regions of the columnar cells.

The epithelium of the posterior part of the oesophagus grades into that of the long hepatic region, where it is somewhat thicker but otherwise very similar, without any sharply defined transition. The hepatic region of the gut (Fig. 8*c*) of this species is remarkable for the total absence of any trace of sacculation of its walls. The lumen is expanded and the gut nearly fills the trunk cavities, its outer surface being almost in contact with the body wall laterally and only separated from it by the gonads dorsally and the muscle bands ventrally.

The intestine is also expanded and has a wide lumen (Fig. 8*d*). The coelomic cavities in both the hepatic and intestinal regions are much narrower than in *S. cambrensis*. The epithelium of the intestine is somewhat thinner than that of the hepatic region, but is thickened ventrally to form two broad convex ridges, one on each side of a mid-ventral groove. The anal region was missing from all the specimens sectioned.

The gonads, both ovaries and testes, are simple unlobed sacs. The more posterior gonads of the series are rudimentary and do not display active gametogenesis. The genital ducts are extremely short, so that the gonads open almost directly at the genital pores. The ovaries (Fig. 7) contain yolk cells and the oocytes were observed to attain a size of 0·23 mm. long by 0·17 mm. broad. Thus, assuming that some of the specimens were mature, the oocytes are considerably smaller than those of *S. cambrensis* and the number present in a single ovary is correspondingly greater.

Comparison with other Species

S. horsti is a well-defined species. It is readily distinguishable from *S. ruber* and *S. serpentinus*, though neither has been described in detail, both by the large number of gill slits and by the concentric arrangement of the musculature of the proboscis. It resembles more closely both *S. cambrensis* and *S. kowalevskyi* and appears to occupy a systematic position intermediate between them, though probably more closely allied to the latter than to the former species. It differs from *S. cambrensis* in coloration, habitat and size; in the more complete concentric arrangement of the longitudinal musculature of the proboscis; the presence of a ventral groove on the proboscis; the straight stomochord; the presence of only four epidermal zones in the collar; the shorter crura of the proboscis skeleton; the slight operculum; the continuity at intervals between the tissues of the neural keel and the nerve-fibre layer of the epidermis; the larger number of gill slits; the projecting ventral muscle bands of the trunk; the genital ridges and extreme anterior extension of the gonads; the form of the epibranchial ridge; the number of intestinal pores. It differs from *S. kowalevskyi* in the presence of dorsal and ventral grooves on the proboscis; the complete separation of the collar cavities; the shorter crura of the proboscis skeleton; the larger number of gill slits; the fact that the tongues project further into the lumen of the pharynx than do the septa; the form of the epibranchial ridge; the less projecting ridges formed by the ventral muscle bands and the gonads.

The structural similarity of *S. horsti* and *S. kowalevskyi* raises the problem of whether Caullery & Mesnil's (1916) record from the coast of France should be ascribed to the British or American species. They had only one fragmentary specimen, which included part of the proboscis, the collar and the anterior part of the branchial region. It was found in very fine and compact grey sand at low-water mark of spring tides. The collar was 3 mm. in diameter and 4–5 mm. long with a marked posterior thickening. There were numerous gill pores which were not covered by lateral folds of the trunk. The proboscis was rosy white in colour and the predominant colour of the rest of the body was orange-salmon. They stated that this coloration was closely similar to that in Spengel's (1893) figure of *S. kowalevskyi*, though not so bright. The musculature of the proboscis was well developed and concentrically arranged.

A single proboscis pore was present on the left side. The proboscis skeleton was similar to that described by Spengel in *S. kowalevskyi*. The perihaemal spaces were fused anteriorly and the muscles in them were much less developed than those figured by Spengel. The branchial epithelium was of the same type as *S. kowalevskyi* and the ectoderm of the collar was very thick with an enormous development of mucus glands. This description is applicable equally to either species, except for the poor development of the muscles in the perihaemal cavities, which conforms with neither. The size, coloration and musculature of the proboscis serve to distinguish it from the other British species, *S. ruber*, *S. serpentinus* and *S. cambrensis*. Thus, while the characters permit of assigning the specimen to either *S. horsti* or *S. kowalevskyi* they do not allow of distinguishing to which it belongs. The geographical evidence points strongly to *S. horsti*, which occurs within 75 miles, whereas all other records of *S. kowalevskyi* are from the other side of the Atlantic Ocean. On this assumption *S. kowalevskyi* falls into line with all other members of the genus in being confined in its distribution.

The known species of the genus *Saccoglossus* now number fourteen. The characters of these, excepting *S. cambrensis* and *S. horsti*, are admirably summarized and a full bibliography of the original literature is provided by van der Horst (1927–39). Nevertheless it may be convenient to include herein a very brief summary in English of the more important and distinctive characters of the species other than that now described.

S. sulcatus (Spengel), from Japan. Deep dorsal sulcus on proboscis rendering it half-moon shaped in cross-section. Collar as broad as long. There appear to be only 10–11 pairs of gill pores. Other characters unknown.

S. otagoensis (Benham), from New Zealand. Deep dorsal groove on proboscis but not so pronounced as in last-named species. Collar as broad as long. 10–15 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Dorsal diverticulum of buccal cavity present. Dorsal and ventral mesenteries of collar absent. Gonads extending anteriorly to level of 4th gill pore. Ventral septum of proboscis short. One pair of intestinal pores.

S. pygmaeus Hinrichs and Jacobi, 1938, from Heligoland. Proboscis without pronounced dorsal groove. Collar broader than long. 9–22 pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. No dorsal diverticulum of buccal cavity. Dorsal and ventral mesenteries of collar complete. Gonads begin at posterior extremity of branchial region. Ventral septum of proboscis very short. One pair of intestinal pores. Very small form, about 3 cm. long.

S. gurneyi (Robinson), from Suez. Collar nearly twice as broad as long. 40–60 pairs of gill pores. Ventral septum of proboscis very long, extending beyond tip of stomochord. Dorsal diverticulum of buccal cavity present. Median proboscis pore. Gonads beginning immediately behind collar. Dorsal and ventral mesenteries of collar complete throughout 4/5ths of length of collar.

S. caraibicus (van der Horst), from West Indies. Collar longer than broad. More than 50 pairs of gill pores. Ventral septum of proboscis long, extending to tip of stomochord. No dorsal diverticulum of buccal cavity. Median proboscis pore. Gonads beginning behind 4th gill pore. Dorsal and ventral mesenteries of collar complete. Perihaemal cavities separate throughout length and not extending into proboscis stalk. Dorsal glomerulus present.

S. bournei (Menon), from Madras. Collar broader than long. More than 62 pairs of gill pores. Ventral septum of proboscis very long, extending in front of tip of stomochord. No dorsal diverticulum of buccal cavity. Gonads beginning immediately behind collar. Dorsal mesentery of collar complete, ventral lacking in anterior half. Very deep anterior neuropore.

S. pusillus (Ritter), from California. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 60 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Ventral mesentery of collar complete. Gonads beginning behind 12th–17th gill pore. One pair of intestinal pores.

S. mereschkowskii (Nic. Wagner), from Northern and Eastern Russia. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 50 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Dorsal mesentery of collar complete, ventral incomplete anteriorly. Gonads beginning in middle of branchial region. Epidermis of collar very thick (0·5 mm.). Seven pairs of intestinal pores.

S. inhacensis Kapelus, 1936, from S. Africa. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 82 or more pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. Ventral septum of proboscis short. Ventral mesentery of collar complete. Gonads beginning at level of 4th gill pore. Four pairs of intestinal pores, the first with several internal openings each.

S. kowalevskyi (A. Agassiz), from Atlantic coast of U.S.A. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 100 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Dorsal and ventral mesenteries of collar incomplete. Gonads beginning within $\frac{1}{2}$ mm. of collar. 4 to 6 pairs of intestinal pores.

S. cambrensis Brambell and Cole, 1939, from Wales. Trunk circular in cross-section, without genital or muscular ridges. 60–90 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Dorsal and ventral mesenteries of collar complete. Gonads beginning in middle of branchial region. S-shaped bend on stomochord in front of ventral diverticulum. 8-12 pairs of intestinal pores, the first 3–5 pairs being rudimentary.

S. ruber (Tattersall), from Ireland. Trunk circular in cross-section, without genital or muscular ridges. 56–64 pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. S-shaped bend on stomochord in front of ventral diverticulum. Ventral septum of proboscis short. Wide proboscis coelom.

S. serpentinus (Assheton), from Scotland. Trunk circular in cross-section, without genital or muscular ridges. 60 pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. Narrow proboscis coelom. Very elongated form, proboscis at least 25 mm. long.

SUMMARY

1. *S. horsti* occurs on the Hampshire coast of the Solent near the mouth of the Lymington River, in glutinous grey mud associated with *Corophium volutator*.

2. The species is distinguished by the following characters: coloration; dorsal and ventral grooves present on the proboscis throughout its length; collar forming a slight operculum posteriorly; ventral muscle bands of the trunk forming projecting ridges; rounded genital ridges present, the gonads beginning within 1 mm. of the collar; 100–140 pairs of gill slits; longitudinal muscle fibres of the proboscis arranged in nine or more concentric rings; ventral septum of the proboscis short; stomochord straight, with ventral diverticulum and wide lumen throughout; no dorsal glomerulus present; single proboscis pore on the left side; proboscis skeleton embracing half to two-thirds of the circumference of the buccal cavity and extending one-fourth to one-third of the length of the collar; four epidermal zones present in the collar; collar cavities completely separated; anterior extensions of the collar cavities extending into the neck of the proboscis; perihaemal cavities separate posteriorly but confluent in front of the level of the tips of the crura, and extending into the neck of the proboscis as far as the proboscis pore; neural keel well developed and in continuity with the epidermal nerve layer at intervals; no dorsal diverticulum of the buccal cavity; branchial epidermis characterized by the predominant intensely eosinophil glandular elements; tongues broader and projecting further into the lumen of the pharynx than the septa; epibranchial ridge formed by two convex ridges bounding a median groove; oesophagus divided into three regions; 4–8 intestinal pores; hepatic region of gut not sacculated and with an expanded lumen; gonads not lobed; oocytes attain a size of 0·23 × 0·17 mm.; yolk cells present in the ovaries.

3. The probable identity of the specimen of *Saccoglossus* from the French coast of the English Channel, recorded by Caullery & Mesnil (1916) as *S. kowalevskyi*, with this species is discussed.

APPENDIX

Analysis of sample of mud, in which *S. horsti* occurs, for which we are indebted to Prof. G. W. Robinson:

Coarse sand (particles 2·0–0·2 mm. in diameter)	0·0
Fine sand (particles 0·2–0·02 mm. in diameter)	39·0
Silt (particles 0·02–0·002 mm. in diameter)	19·7
Clay (particles less than 0·002 mm. in diameter)	30·1
Moisture in air-dry sample	3·2
Calcium carbonate	1·6
Organic carbon	2·5

The sample was oven dried at 105° C. before analysis. Comparison with the analyses of sand in which *S. cambrensis* occurs (Brambell & Cole, 1939*a*) shows that this sample contains a much higher proportion of silt and clay and that the organic content is considerably greater.

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THE MALE OF THE AMPHIPOD *HAUSTORIUS ARENARIUS* SLABBER

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The amphipod *Haustorius arenarius* has been recorded by several workers as an inhabitant of the intertidal and shallow-water sands of the coasts of Britain, its characteristic shape being regarded as an adaptation to its habit of burrowing in the sand. Sars (1895) describes the female only, and Stebbing (1906) summarizes the description given by Sars. Chevreux & Fage (1925) record the species from numerous localities on the coast of France and give in addition the Kattegat, Holland, Great Britain and the east coast of North America as the extent of its distribution. They state that the male is unknown. This apparent absence of the male of the species formed a notable exception amongst the Amphipoda, a group in which the males may usually be distinguished from the females by a number of secondary sexual characters of which the brood lamellae of the adult female is the most characteristic.

Sexton (1925), in her study of the growth, moulting and mating habits of species in the genus *Gammarus*, has shown that the brood lamellae develop gradually, increasing in size through a series of moults and finally becoming fully formed by the development of long fringing hairs. Once formed the lamellae remain a constant feature at each moult. Hart (1930) shows that such a mode of development also occurs in *Corophium volutator*. Unwin (1920) has shown that in the isopod *Asellus aquaticus* the brood lamellae appear suddenly at the ecdysis accompanying fertilization and disappear at the moult which succeeds the release of the young from the pouch. Sheppard (1927) draws attention to this distinction between *Asellus* and *Gammarus* as one which may prove to be constant throughout the two groups.

Dennell (1934), in his study of the habits and feeding mechanism of *Haustorius arenarius*, states (p. 374): 'Of the 120 specimens examined all were females possessing oostegites, or lacking them, but resembling those so provided in all other respects'; and also p. 375, 'Whether reproduction takes place parthenogenetically or whether there may be a male of the species is unsettled'.

These remarks of Dennell suggested to me that the 'females lacking oostegites' were males. To test this assumption a collection of *H. arenarius* from the estuary of the river Dovey was examined and grouped on the presence or absence of brood lamellae. Of the fifty individuals which formed the sample twenty-five showed brood lamellae in various stages of development as described by Sexton in the genus *Gammarus*. This group varied in total length from 5 to 14 mm., and a number of those 11 mm. and over carried eggs in the brood pouch. Of the remaining twenty-five, varying in length from 5 to 10 mm., a number were dissected and sectioned. All showed a male reproductive system which, as is usual in amphipods, lay dorsal to the alimentary canal and its associated diverticulae. The system consists of an anterior testis portion which narrows to a vas deferens on which a swollen receptaculum seminis occurs which opens at the base of the seventh segment of the mesosome.

This sample was collected in July. A further sample collected in December showed the same general features except that no egg-bearing females were obtained and the brood lamellae were without the fringing hairs. In each case no individual less than 5 mm. in length was obtained. A collection of *Haustorius arenarius* from Kames Bay gave individuals of 3 and 4 mm. total length, none of which size bore developing brood lamellae. It thus appears that the brood lamellae begin to form at a size of about 5 mm.

A comparison of the appendages of the adult males and females, particularly of those which show secondary sex differences in other amphipods, namely, the antennules and antennae, the first and second gnathopods and the third uropods, failed to show a single morphological difference. The related genera in the family Haustoriidae, *Bathyporeia* and *Urothoë*, show a secondary sex difference in the length of the flagellum of the antenna which is long and bears calceoli in the male and is short and without calceoli in the female. No such distinction occurs in *Haustorius*. Sexton & Spooner (1940) have shown that in the genus *Marinogammarus* the males bear special sensory setae, particularly on the antenna. I was unable to distinguish any differences in the setal armature of the male and female of *Haustorius*.

SUMMARY

The male of *Haustorius arenarius* is identical with the female in all external morphological characters apart from the absence of brood lamellae. The females develop brood lamellae at 5 mm. total length, eggs appear in the brood pouch at 11 mm., and they may reach a length of 14 mm. The largest male obtained measured 10 mm.

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ON THE LIFE HISTORY AND DEVELOPMENTAL STAGES OF THE MEDUSA *PODOCORYNE BOREALIS*

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(Text-fig. 1)

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INTRODUCTION

It has been shown in a recent paper by Russell (1940) that in addition to *Podocoryne carnea* M. Sars, 1846, and the so-called *P. areolata*, two other *Podocoryne* medusae, viz. *P. hartlaubi* Neppi & Stiasny, 1913, and *P. minima* (Trinci), also occur in the plankton in British waters. The identity of both hydroid and medusa of the European[1] *P. carnea* is well established and the medusa has never been known to mature with more than 8 tentacles (Kramp, 1927; Russell, 1940). *P. minima* is a small medusa with only 4 tentacles and need not be considered further in this paper.

As there were only two species of medusae of the genus *Podocoryne* previously known from British waters, it was generally assumed that the newly liberated medusa of the hydroid *P. areolata* (liberated with 16 tentacles) was the earliest stage of the medusa found in the plankton with 16–32 tentacles. The discovery by Russell of a second species in the plankton (*P. hartlaubi* with 24–49 tentacles) indicated that the hydroid *P. areolata* might belong to either species.

While at Millport during 1940 I obtained a fine series of all stages of the so-called *P. areolata* in the plankton. The earliest stage of the medusa has only 8 tentacles, so that it cannot belong to the hydroid *P. areolata*. As will be shown below, the youngest stages may have been confused in the past with *P. carnea*. Kramp & Damas (1925) have regarded the mature medusa as identical with *Lymnorea borealis* Mayer, 1900. They have also re-examined the species described by Broch (1905) as *Limnorea norvegica* and identified it as *P. areolata*. Since this last specific name can no longer be used for this medusa, I propose to refer to the species as *P. borealis*. There is some evidence to suggest that a *Podocoryne* hydroid which I found in the Clyde belongs to this species.

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[1] The supposed occurrence of *P. carnea* in North American waters with more than 8 tentacles is discussed on p. 315.

The first certain records of this medusa from British waters are those of Browne (1895, 1897) as ?*Cytaeandra areolata* from Port Erin and Valentia. Hartlaub (1911) gives a full list of British and northern records of the medusa, but his records of the hydroid are those of *P. areolata* (Alder). Previous records of the medusa from Millport are given by Browne (1905), who states that in 1901 it occurred in April and July; in 1902, young stages were found in March and adult medusae in May. During 1940, young stages were found occasionally in the plankton from the beginning of March to the end of September, while the earliest mature medusae were present on 2 May. The young stages obtained throughout this period indicate that the hydroid is fertile during the spring, summer and autumn.

All the material of *P. borealis* was collected at Millport while I was holding a grant from the Royal Society. I wish to thank Dr Stanley Kemp, F.R.S., for facilities at Plymouth where the paper was completed.

THE HYDROID

A well-developed colony of a hydroid, which appeared to be identical with, and was at first thought to be, *P. carnea*, was found on a dead *Buccinum* shell dredged off the North End of Cumbrae on 6 March 1940. When found, all the polyps were large and particularly well developed as in mature colonies of *Hydractinia echinata*, and some of them bore medusa buds on the body of the hydranth. On the fertile polyps, which possessed 9–14 filiform tentacles, there were from three to eight medusa buds. The polyps arose from a creeping network of stolons and there was no encrusting base or spines; the absence of the latter, however, might depend on the age of the colony. Medusae were liberated on the following day in the laboratory and these possessed better developed interradial tentacles than I had ever seen in specimens liberated from the true *P. carnea*. In all the young specimens liberated all 4 interradial tentacles were present. After many days of rearing the young medusae had developed no gonads on the stomach. Suspicions that this hydroid was distinct from *P. carnea* were confirmed when I found a complete series of twenty medusae, from the newly liberated to the sexually mature with 20–31 tentacles, in the plankton on 2 May 1940 (see Table I). The youngest specimens were obviously newly liberated and agreed in all points with my specimens from the hydroid. The hydroid had been many days in captivity before it was realized that it was distinct from *P. carnea*, and by this

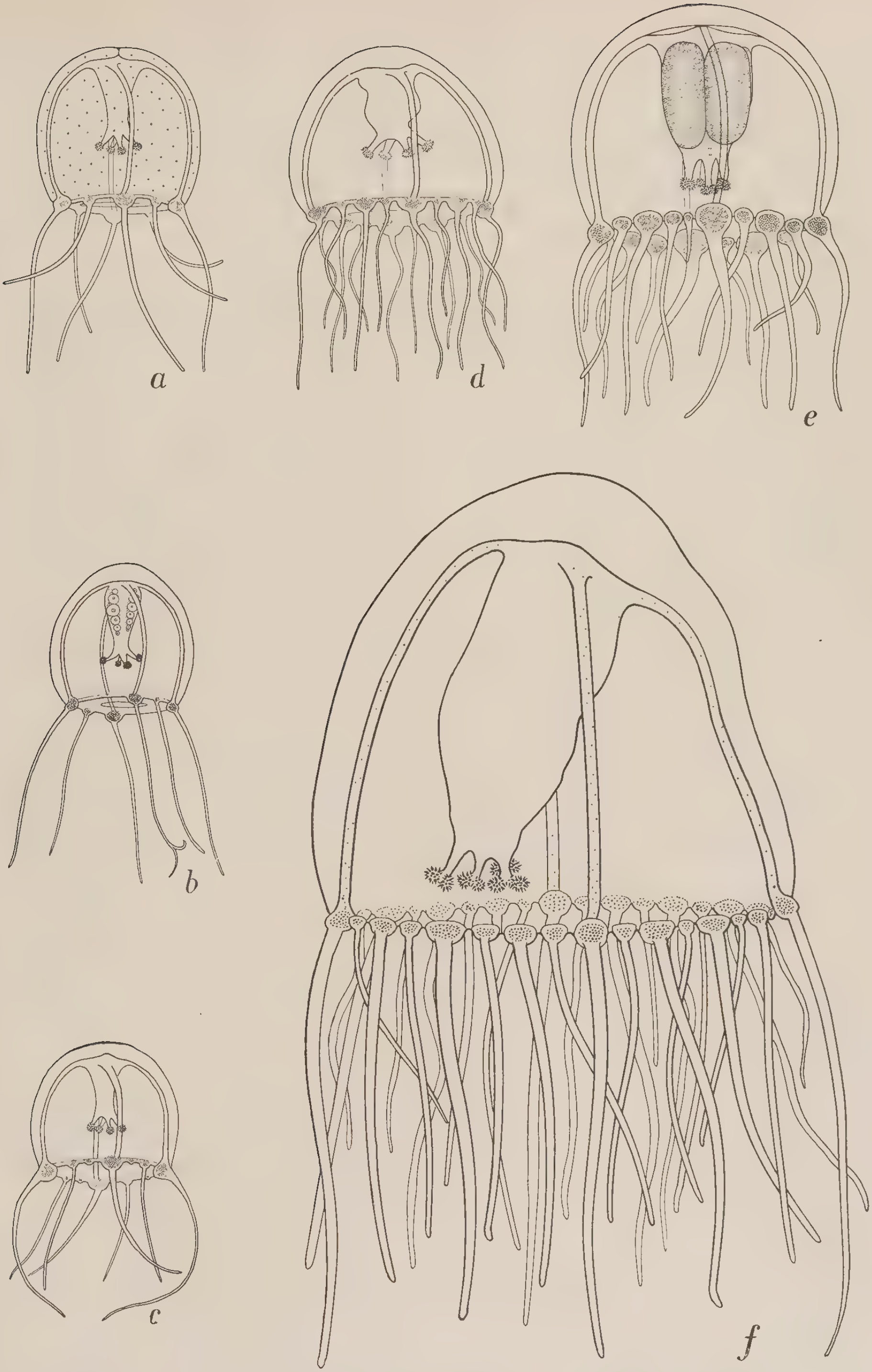

Fig. 1. *a, Podocoryne borealis*; medusa newly liberated from the hydroid, Millport, 7. iii. 40. *b, P. carnea*; mature female medusa reared from the hydroid, Plymouth, 2. x. 36. *c, P. borealis*; medusa, 8 tentacle stage, Millport, 6. v. 40. *d, P. borealis*; medusa, 16 tentacle stage, Millport, 6. v. 40. *e, P. borealis*; medusa, with 17 tentacles, mature male, Millport, 6. v. 40. *f, P. borealis*; mature female medusa with 31 tentacles, Millport, 2. v. 40.

time all the fertile and sterile polyps had become considerably reduced by the reproductive activity of the hydroid. A full description of the colony at this stage might give a misleading picture of the hydroid and it is hoped later to re-describe the species from fresh material.

THE NEWLY LIBERATED MEDUSA (Fig. 1*a*)

Medusae newly liberated from the hydroid in the laboratory were 0·8–1·0 mm. high and 0·9–1·2 mm. in diameter. They were of a deep bell-shape with a uniformly thin umbrella with ex-umbrellar nematocysts and a well-developed velum. The stomach was tubular and in length about half the height of the sub-umbrellar cavity. There were four distinct mouth lips, each terminating in a knob of nematocysts. The radial canals were prominent and united around the margin by a ring canal. There were four large perradial tentacular bulbs, 0·1 mm. in diameter, each with a well-developed tentacle. Interradially, there were four smaller bulbs each with a shorter tentacle than the perradial tentacle. The base of the stomach and the tentacle bulbs were of a brownish colour. Of twenty-eight medusae liberated from the hydroid described on p. 308, all possessed 8 tentacles, 4 of which, the perradial tentacles, were long and of equal length, while the interradial tentacles were a little shorter. There was no trace of gonad in any of the medusae even after many days of rearing.

These medusae may be compared with the newly liberated ones obtained from *P. carnea* which I found on a shell of *Nassarius reticulatus* dredged in Cawsand Bay, Plymouth, on 14 September 1936. Those liberated between 21 and 26 September differed but little in size, colour and the diameter of the tentacle bulbs from *P. borealis*, but there was considerable variation in the development and number of the interradial tentacles. Some possessed none. Browne (1896) also noticed the variation in the number of interradial tentacles and states that he did not see a specimen with 8 tentacles amongst those reared from the hydroid in his jar. Forty of the fifty-one specimens examined possessed immature gonads. The variation in the development of the interradial tentacles is tabulated below.

	No. of medusae	No. of perradial bulbs	No. of interradial bulbs
21. ix. 36	1	4	2 less developed, 1 rudiment
23. ix. 36	1	4	4 less developed
25. ix. 36	7	4	4 less developed
26. ix. 36	10	4	None
	10	4	2 less developed
	10	4	2 fully and 2 less developed
	12	4	4 fully developed

A number of these were kept for rearing, and after a few days some of them became sexually mature (Fig. 1*b*). The specimen figured matured in a few days with 4 perradial tentacles and 2 interradial tentacles. The perradial tentacular bulbs of this specimen had a diameter of 0·18 mm.

Stages from the Plankton

The earliest stages from the plankton differed in no way from the medusae obtained in the laboratory and were 0·65–1·0 mm. high and 0·65–1·1 mm. in diameter. Here the tentacles are still unequal in length and may be referred to as 4+4. In the descriptions below, the smallest tentacles are indicated in this way. Rudimentary bulbs from which no rudiment of a tentacle has developed are not included in the total. The succeeding stages can be roughly grouped into 16, 24 and 32 tentacle stages, but these are not very well defined. The tentacles themselves vary in length; for example, in the 8 tentacle stage described below, the interradial bulbs and tentacles are smaller than the perradial ones, while the rudimentary tentacular bulbs of an additional 8 tentacles are already appearing. Thus new tentacles are appearing even before those formed earlier reach full size. Even at the 16 tentacle stage and in older specimens the interradial tentacular bulbs have a slightly smaller diameter than the perradial bulbs. The differences in the sizes of the tentacular bulbs, however, do not appear to be as well marked as in *P. hartlaubi.*

The 8 *Tentacle Stage* (Fig. 1*c*)

A medusa typical of this stage was captured on 6 May. The umbrella was 1·1 mm. high and 1·2 mm. in diameter. It had scattered nematocysts on the ex-umbrella. The stomach had a length of 0·8 mm., reaching a little more than half-way to the margin of the bell. The mouth had four simple lips each terminating in a round cluster of nematocysts. There were 8 tentacles, the 4 interradial ones being well developed. The perradial and interradial tentacular bulbs had a diameter of 0·2 and 0·4 mm. respectively. Eight additional bulbs were developing on the margin of the bell, but these were rudimentary bulbs without tentacles. All the bulbs were of a brownish colour. There was no trace of developing gonads. Kramp & Damas (1925) describe a small specimen 1·25 mm. in height which appears to belong to this stage. It has 8 tentacles (4+4) and 8 adradial tentacular rudiments. An apical canal was present and on account of this they regarded it as newly liberated. In my specimens an apical canal was rare, but when present it persisted for a long time. Kramp & Damas also describe the oral lips as undivided in young examples.

The 16 *Tentacle Stage* (Fig. 1*d*)

I have many intermediate stages between the 8 tentacle stage and the 16 tentacle stage. It is at this latter stage that the rudiments of gonads begin to appear. The following description is of an immature female specimen obtained on 6 May (Fig. 1*d*). The umbrella was 1·7 mm. high and 1·8 mm. in diameter. There were 16 tentacles, the perradial and interradial bulbs being much better developed than the adradial bulbs. The perradial, interradial and adradial bulbs had diameters of 0·28–0·3, 0·2–0·22 and 0·1 mm. respectively.

There were no rudimentary bulbs and the tentacles were more uniform in length than in many similar stages. The stomach was a little contracted and had a slight peduncle. The batteries of nematocysts on the four lips of the mouth were oval in shape, 0·08 × 0·12 mm.

Some medusae at this stage possess immature gonads and frequently become mature by the time a few more tentacles are added. Fig. 1*e* is a drawing of a mature male medusa with 17 tentacles (16 + 1). The majority of the sexually mature specimens that I collected, however, possessed 20–24 tentacles, while a few large specimens had 30–32 tentacles.

A 16 tentacle stage has been figured by Browne (1897, Pl. xlviii, fig. 1) as ?*Cytaeandra areolata* Haeckel. It is an earlier stage than Fig. 1*e* and is immature. The constriction at the base of the stomach in Browne's figure is more pronounced than in my specimens. Mayer (1910, Pl. 14, figs. 4, 5) figures two 16 tentacle stages similar to mine as *P. carnea*; there is no doubt that they belong to the present species.

The 24 *Tentacle Stage*

Mature and immature medusae were found at this stage and at intermediate stages between this and the 16 tentacle stage. In all the 24 tentacle stages found the mouth lips on the stomach were divided, possessing eight nematocyst knobs. An immature medusa belonging to this stage has been described and figured by Browne (1897, Pl. xlviii, fig. 2) from material obtained at Valentia, Co. Kerry. He also notes the occurrence of a female medusa with 24 tentacles and bearing ova on the stomach.

The 30–32 *Tentacle Stage* (Fig. 1*f*)

The biggest medusa caught in the series obtained on 2 May 1940 belonged to this stage. This specimen (Fig. 1*f*) was a ripe female medusa having a height of 3·55 mm. and a diameter of 4·0 mm. The umbrella was thickened at the apex and gradually thinned towards the margin of the bell. There were 31 tentacles (30 + 1) and one tentacular bulb without a tentacle. The perradial, interradial, and adradial tentacles were better developed than later developed tentacles. The perradial, interradial and adradial tentacular bulbs had diameters of 0·35, 0·28–0·3 and 0·3 mm. The tentacles, too, showed much variation in length. The radial canals had a diameter of 0·1 mm. The stomach was long and tubular, reaching nearly to the margin of the bell. The four mouth lips and the nematocyst knobs had become completely divided, so that there were eight distinct nematocyst knobs: in the preserved specimen as drawn the lips are contracted and do not show this well. These branched lips during life had the same appearance as those figured by Browne (1897, Pl. xlviii, fig. 2) in a specimen with 25 tentacles. In this and other well-developed specimens, the nematocysts formed a distinct continuous band along the edges of the lips from knob to knob. The nematocysts were of the spindle shape figured by Mayer (1910) and Allman (1871–2). The four gonads were interradial in position and occupied the greater part of the length of the stomach and extending to meet each other in the perradii. The mature eggs on the interradii were dark and granular in appearance, while the more immature towards the edge of the gonad were transparent, giving, as Mayer states in his description of *Lymnorea borealis*, a reticulate appearance to the surface of the gonad. In the immature eggs the nuclei, each with a nucleolus, were plainly visible under the low power of the microscope.

The stomach, radial canals and ring canal were a delicate pinkish red in colour, while the endoderm of the tentacle bulbs was of a deep reddish-brown colour giving the living medusa a handsome appearance. During life, when the medusa was propelling itself through the water, the tentacles were frequently coiled in tight spirals.

Another mature stage with 29 tentacles, caught on 3 October 1940, had more fully branched oral lips which were twice dichotomously branched, so that there were sixteen nematocyst knobs. This is the condition figured by Mayer (1910, Pl. 15, figs. 1–3) for *Lymnorea borealis*.

Additional data and measurements of a fine series of medusae caught in a single haul from Keppel Pier on 2 May 1940 are given in Table I. The youngest specimen was a newly liberated specimen, while all the specimens with more than 20 tentacles were sexually mature.

Discussion

The discovery of the various stages in the life history raises the question of what name the species should bear, since it can no longer be called *Podocoryne areolata*. Mayer stated his belief that the *P. areolata* of British waters might prove to be identical with his *Lymnorea borealis*. It is well known that the oral lips of the manubrium are branched in *P. areolata*, and it has been shown in this paper that the lips may branch in the larger specimens, so there is now no doubt that my species from Millport is identical with *L. borealis*. Mayer distinguished the genus *Podocoryne* from *Lymnorea* by its simple oral lips, but in this species the distinction only holds good for the youngest stages, and I have therefore referred the species to *Podocoryne* as *P. borealis*.

It will be seen from the foregoing description that three species are associated with the history of this species and may have been confused with it in the past. These are *P. carnea* M. Sars, 1846, *P. areolata* (Alder, 1862) and *P. hartlaubi* Neppi & Stiasny, 1913.

The European *P. carnea* is well known and the first description of the hydroid and young medusa by Michael Sars undoubtedly refers to the species now generally recognized under that name, that is, with the medusa maturing with 8 tentacles. Lovén (1857) also recorded this medusa from Bohuslans in Norway and was able to obtain sexually mature medusae with 8 tentacles. Kramp (1927) states that 'no specimens of this medusa have ever been taken

TABLE I. *PODOCORYNE BOREALIS* (MAYER). SERIES OF MEDUSAE CAUGHT AT KEPPEL PIER, MILLPORT, 2 MAY 1940. MEASUREMENTS IN MM.

Specimen no.	Diameter	Height	No. of tentacles	Length of stomach	Diameter of per-radial bulbs	Sex	No. of nematocyst knobs on oral lips	Remarks
1	0·85	0·95	8 (4+4)	0·4	0·13	—	4, round	Newly liberated
2	1·2	1·5	16 (8+8)	0·6	0·22	—	4, round	Young specimen
3	1·7	1·45	16 (8+8)	0·8	0·2	—	4, round	Fairly young specimen
4	1·1	1·3	16 (8+8)	1·0	0·2	—	4, round	Stomach full
5	1·7	1·6	16 (12+4)	1·2	0·25	Imm. male	4, oval	Stomach reaching bell margin
6	1·9	1·6	16 (8+8)	1·0	0·26	?Imm. female	4, oval dividing	Gonads just appearing
7	2·0	1·6	16 (8+8)	1·4	0·2	Imm. male	4, oval	Stomach reaching bell margin
8	2·2	2·3	16 (8+8)	—	0·26	Imm. male	—	Mouth and part stomach missing
9	2·1	2·0	16 (12+4)	1·45	0·24	Imm. male	4, oval	Stomach nearly reaching margin
10	2·1	2·1	19 (16+3)	1·05	0·25	Imm. female	4, all dividing	Stomach reaching half-way to margin
11	2·1	1·8	16	1·45	0·3	Male	4, 2 oval, 2 dividing	Stomach reaching bell margin
12	2·15	2·0	19 (16+3)	1·3	0·25	Imm. female	4, 2 oval, 2 dividing	Gonads just appearing
13	2·6	2·15	21 (17+4)	—	0·22	Male	—	Mouth and part stomach missing
14	2·8	2·2	20 (16+4)	1·3	0·3	Imm. female	4, all dividing	The 4 small tentacles are very small
15	2·65	3·0	20 (16+4)	1·5	0·33	Male	4, all dividing	Gonads mature
16	2·4	2·0	20 (16+4)	1·45	0·25	Male	8 distinct knobs	Gonads mature
17	3·0	2·7	25 (22+3)	1·85	0·32	Male	8 distinct knobs	Gonads mature
18	3·0	3·0	24 (19+5)	2·2	0·3	Male	8 distinct knobs	Gonads mature
19	3·4	2·6	26 (22+4)	1·8	0·3	Male	8 distinct knobs	Gonads mature
20	4·0	3·55	31 (30+1)	3·0	0·35	Female	8 distinct knobs	Gonads mature

in north European waters with more than eight marginal tentacles'. Russell (1940) makes a similar statement. There is, however, a very doubtful record of a specimen with 16 tentacles from Trieste by Graeffe (1884). Mayer (1910) has described *P. carnea* from North American waters maturing with 8, 16, 24 or 32 tentacles, and because of the numerous tentacles has referred to his species as *P. carnea* var. *americana*.

If we were to accept the view that Mayer was dealing with a single species, then my specimens from the Clyde would have to be referred to *P. carnea*, and we would expect to find mature medusae with 10, 12, 14 or 15 tentacles in the plankton. However, none has ever been recorded in north European waters and we are left with the view that there are two separate species—one medusa maturing with 8 tentacles and the other maturing with 16–32 tentacles. The close similarity of the two hydroids and an apparently continuous series of mature medusae at the 8, 16, 24 and 32 tentacle stages may have led Mayer to assume that he was dealing with a single species. There is nothing in Mayer's account to suggest that he obtained mature medusae at intermediate stages between the 8 and 16 tentacle stages. A careful study of Mayer's description of the hydroid shows that in all probability he was dealing with two distinct species—one of which was identical with the European *P. carnea* (Mayer, 1910, text-fig. 74) and the other was *P. borealis* (Pl. 14, fig. 2). He states that 'the reproductive polypites, or gonostyles, are frequently exactly similar in size and shape to the feeding-polypites, and, in fact, are probably merely feeding-polypites which have developed medusa buds (see Pl. 14, fig. 2, *g*). In other instances the gonostyles are smaller and more slender, and possess not more than 4 to 8 tentacles.' In the hydroids of *P. carnea* which I have been able to collect at Plymouth the reproductive polyps have always been small with only 4 or 5 tentacles. In the hydroid from Millport, however, the reproductive polyps were as large as the sterile ones and only later diminished in size after liberating numerous medusae. Allman (1871–2) and Hincks (1868) have probably described the two species under the name *P. carnea*, and a careful redescription of both hydroids from ample material is much to be desired. It is thus clear that some at least of the records of the hydroid and young medusa of *P. carnea* are records of *P. borealis*.

The hydroid *P. areolata* (Alder) may be distinguished from other known British species of the genus by the irregular grouping of the chitinous spines on the basal crust and by the position of the medusa buds; these are sessile on the encrusting base. Further, when the medusa is liberated it has 4 large perradial tentacles and 12 smaller intermediate ones, making a total of 16 tentacles. It is, perhaps, because this species has always been associated with the medusa *P. borealis* in British waters that all hydroids of this kind in which the medusa buds originate on the body of the polyps have been assigned to *P. carnea*. Russell (1940) has suggested that *P. hartlaubi* may be the medusa of Alder's species.

Russell has also indicated that there is a possibility that *P. hartlaubi* may have been previously recorded from British waters as '*Podocoryne areolata*' because of its superficial similarity to that medusa.

It is suggested that the synonymy of *P. borealis* and *P. areolata* becomes:

Podocoryne areolata (Alder, 1862):
  Hydractinia areolata, Alder, 1862.
  Rhizocline areolata, Allman, 1864.
  Podocoryne areolata, Hincks, 1868.
  Cytaeandra areolata, Haeckel, 1879.

Podocoryne borealis (Mayer, 1900):
  Cytaeandra areolata, Browne, 1895.
  ?*Cytaeandra areolata*, Browne, 1897.
  Podocoryne areolata, Mayer, 1910; Hartlaub, 1911 (medusa only).
  Lymnorea borealis, Mayer, 1900.
  Limnorea norvegica, Broch, 1905.

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OSMOREGULATION IN SOME PALAEMONID PRAWNS

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(Text figs. 1–8)

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INTRODUCTION

In recent years much progress has been made in our knowledge of osmotic changes in aquatic organisms, chiefly in marine animals that can successfully withstand a certain amount of dilution of the external medium. In addition to confirming the classical findings of Bottazzi (1897, 1908) that, unlike the teleosts, the body fluids of marine invertebrates and elasmobranchs are isotonic to the sea water in which they live, investigations by numerous workers have shown that the colonization of fresh and brackish waters by marine animals is closely dependent upon the development of osmoregulatory powers which attain a high degree of perfection in freshwater organisms. Schlieper and his school (Schlieper, 1929, 1930, 1935; Schlieper & Herrmann, 1930; Schwabe, 1933; Scholles, 1933; Peters, 1935) brought evidence of the relative importance of the excretory organs in osmoregulation; this has been confirmed by other investigators who have also emphasized the question of differences in permeability. Finally, the discovery of Krogh (1937, 1938) that many animals in fresh water are capable of actively absorbing ions from their surroundings, even when the latter are present only in exceedingly minute quantities, has introduced a new aspect to the problem of adaptation of marine animals to fresh and brackish waters.

One would normally expect a marine invertebrate to be isotonic with its medium when in sea water, and it was therefore of unusual interest to find two crustaceans in the sea-water tanks of the Plymouth Laboratory showing a blood concentration markedly hypotonic to the external medium. One is the widely distributed and well-known brackish-water prawn *Palaemonetes varians* (Leach) (Panikkar, 1939) and the other the common marine prawn *Leander serratus* (Pennant) (Panikkar, 1940*a*). A similar behaviour was also later discovered in *Leander squilla* (L.). The difficulty these animals have to overcome is not that of maintaining a higher concentration of blood in an external medium of low concentration, which we find in most estuarine animals, but of keeping the blood dilute in defiance of the more concentrated external medium and the consequent prevention of loss of water from the tissues. This condition, however, changes when these same species are in brackish or fresh water, where the body fluids have to remain in a state of definite hypertonicity. The mechanism of osmoregulation needs to be equally efficient in maintaining a stable hypotonicity when in sea water and hypertonicity when in fresh or brackish water.

Among marine invertebrates, the few known instances of slight hypotonicity when in sea water are some of the grapsoid and ocypodid crabs (Edmonds, 1935; Baumberger & Olmsted, 1928; Schwabe, 1933; Conklin & Krogh, 1938; Pora, 1939; Pearse, 1932) which belong to groups that have become semi-terrestrial in habits (Table I). Other parallel instances are the highly specialized brine shrimp *Artemia* (Medwedewa, 1927; Kuenen, 1939), the insects that can inhabit salt water (Beadle, 1939; Claus, 1937; Wigglesworth, 1933, 1938), and certain permanently attached parasites that feed entirely on hypotonic blood (Panikkar & Sproston, 1941).

The general condition observed in these prawns is similar to that of the marine teleosts, which are always hypotonic in sea water and hypertonic in fresh. The peculiar osmotic behaviour in the prawns here described becomes still more significant when the habits and distribution of the group to which they belong are considered. *Palaemonetes varians* is known to exist in two distinct varieties, a freshwater and a brackish-water one, confined respectively to the southern and north-western parts of Europe; the widely distributed genus *Leander* includes marine, estuarine and freshwater species. Can we correlate their osmotic behaviour with their plasticity of habits and the ability of certain palaemonid prawns to migrate into fresh water? It seems reasonable to assume that the osmotic independence shown by *L. serratus* and *L. squilla* must have been acquired in fresh water at some period in their evolutionary history, and, if this assumption is correct, we are dealing with a remarkable

TABLE I. CRUSTACEA WHICH ARE HYPOTONIC IN SEA WATER OR CONCENTRATED SALT WATER*

Species	Blood			External medium			Author	Date	Remarks
	Δ – ° C.	mM.	% NaCl	Δ – ° C.	mM.	% NaCl			
Crustacea: Phyllopoda									
Artemia salina	0·70	205	1·20	2·75	804	4·70	Medwedewa	1927	Barger's method. Values for external medium only approximate
	0·76	222	1·30	4·85	1420	8·30			
	0·70–0·82	205–240	1·2–1·4	3·39	992	5·80	Kuenen	1939	Barger's method
	1·28–1·52	376–445	2·2–2·6	10·16	2976	17·40			
Crustacea: Copepoda									
Lernaeocera branchialis	1·17–1·63	342–479	2·0–2·8	1·97–2·04	577–599	3·4–3·5	Panikkar & Sproston	1941	Vapour pressure of blood of ♀ which is permanently attached to fish. Subject to considerable variation
Crustacea: Decapoda									
Palaemonetes varians	1·28–1·40	376–411	2·2–2·4	1·93–2·04	565–599	3·3–3·5	Panikkar	1939	Vapour pressure method. Details in this paper
	1·53	450	2·63	2·79	817	4·78			
Leander serratus	1·52–1·70	445–498	2·6–2·9	1·93–2·04	565–599	3·3–3·5	Panikkar	1940	Vapour pressure method. Details in this paper. Also confirmed by freezing-point method
Leander squilla	1·46–1·52	428–445	2·5–2·6	1·93–2·04	565–599	3·3–3·5	Panikkar	1941	Vapour pressure method. Details in this paper
Grapsus grapsus	1·92	563	3·29	2·04	599	3·5	Pearse	1932	Freezing-point
Pachygrapsus marmoratus	2·01–2·11	589–618	3·4–3·6	2·20–2·33	645–683	3·8–4·0	Schwabe	1933	Freezing-point. Also confirmed by Pora (1939)
Pachygrapsus crassipes	1·32	387	2·3	1·97	577	3·4	Baumberger & Olmsted	1928	Freezing-point. Hypotonicity disappears in certain stages of moult cycle
Leptograpsus variegatus	1·95	571	3·3	2·13	624	3·6	Edmonds	1935	Freezing-point
Heloecius cordiformis	2·85	835	4·9	3·40	996	5·8	Edmonds	1935	Freezing-point
	1·95	571	3·3	2·17	636	3·7			
Eriocheir sinensis	1·85–2·05	541–600	3·2–3·5	2·20–2·28	659–667	3·8–3·9	Conklin & Krogh	1938	Vapour pressure method. Hypotonicity apparent only in concentrated sea water
	2·32	679	4·0	2·54	744	4·4			
Ocypoda albicans	1·70	498	2·9	2·04	599	3·5	Pearse	1932	Land crabs. Freezing-point method
Cardisoma guanhumi	1·66	486	2·8	2·04	599	3·5	Pearse	1932	
Gecarcinus lateralis	1·65	483	2·8	2·04	599	3·5	Pearse	1932	

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* Values of Δ and mM. given in this table are based on the relationship that 0·293 mol. Na (or Cl) gives a depression of freezing-point of – 1·0° C.

group of crustaceans which had at one time become accommodated to life in fresh water and has since returned to a marine habitat. The osmotic adaptation involved is of interest because its object is the converse of what is seen in euryhaline animals. The present paper is an attempt to elucidate this osmoregulatory mechanism as studied by changes in total osmotic pressure of blood and urine, and deals with the problem as viewed from the general biological standpoint; the question of ionic regulation will be dealt with in a subsequent paper.

Material and Methods

The prawns used in this investigation were obtained at Plymouth, *Leander serratus* from the Sound, *L. squilla* from Laira, and *Palaemonetes varians* from Chelson Meadow. They were stocked in circulation tanks. No special attempt was made to feed *Palaemonetes*, but *Leander* was now and then fed with bits of *Sepia* and mussels. Judged by the comparatively low mortality among prawns kept in tanks it may be concluded that they remain in a healthy state under circulation. For experimental purposes they were transferred to different media prepared from clean sea water, aerated and kept in large glass jars. The tank water is more saline than outside sea water and has an osmotic pressure of 3·3–3·5 % NaCl; it has a slightly higher calcium content (0·015 *M* as against 0·0098 *M* for outside sea water (Cooper, 1932)). The *p*H is about 8·0, but is subject to slight variation (Atkins, 1922). Dilutions of sea water were always made with Plymouth tap water, which is a very soft water with a slight trace of chloride and about 3 parts per million of silica. Experimental animals were not fed; nor were the jars aerated after the animals had been put into them. Artificial sea water was made with 0·6 *M* NaCl, 0·6 *M* KCl, 0·4 *M* $CaCl_2$ and 0·4 *M* $MgCl_2$ solutions mixed in the proportion of 801·9, 17·2, 27·2 and 139·6 ml. respectively, along with 2 drops of *M* $NaHCO_3$ for every 100 ml. of the mixture, and brought to *p*H 8 with NaOH. Concentrated sea water was prepared by the addition of Tidmann's sea salt to clean sea water, care being taken to bring the *p*H to normal by appropriate buffering.

Estimations of osmotic pressure were made by Baldes's modification of the Hill thermo-electric technique (Baldes, 1934; Hill, 1930, 1931). The standard solutions employed for comparing the vapour pressures were over a fairly wide range, i.e. between 1·8 and 2·5 % NaCl for the blood of *Palaemonetes* and between 2·0 and 3·0 % NaCl for that of *Leander*. After mounting the solutions on the thermocouples in a moist chamber, they were equilibrated in a thermostat kept constant at 20·8° C. ± 0·001, and the deflexions taken from a Downing (moving-coil) galvanometer of sensitivity $1·28 \times 10^{-8}$ V./mm. at a distance of 3 m. Using tested thermocouples, 1 mm. deflexion on the scale was equivalent to an e.m.f. caused by solutions differing in strength by 0·005 % NaCl, so that, using a magnifying glass, differences up to half the above figure could be read on the scale. All estimations were made in duplicate on two separate instruments, and most of the readings were also taken in the same instrument with the unknown sample placed in a 'normal' and a 'reversed' position. The data given in the tables and graphs are the mean values of the readings taken with each sample. The values of osmotic pressure are throughout expressed as percentage of sodium chloride, i.e. the grams of NaCl dissolved in 100 g. of distilled water to make a solution having the same vapour pressure as the unknown at the same temperature and atmospheric pressure. Owing to variations in the salinity of the tank water, parallel estimations of osmotic pressure of tank water were made in all cases where the animals were taken from the circulation. The experimental solutions used as external media were measured in the same manner as blood and urine, but the standard solutions employed ranged from distilled water to 3·5 % NaCl; special attention was paid to having the filter paper on the walls of the couple chamber moistened with the solutions being compared, to ensure greater accuracy (cf. Hill, 1931). In all experiments the thermocouples were calibrated both before and after a series of estimations, and the mean values taken for final calculations.

The following uniform procedure was adopted for the removal of blood from prawns. The animals were wiped with a dry towel, and the water in the gill chambers and between the cephalothoracic shield and the first abdominal segment was completely removed with strips of filter paper. A capillary pipette (specially drawn out from glass tubing cleaned in bichromate sulphuric mixture and distilled water, and kept in readiness in a desiccator) was introduced into the heart at the point where it is easily accessible from outside, i.e. under the hind-margin of the cephalothoracic shield. The blood collects in the tube because of the hydrostatic pressure and capillarity, but if larger quantities were required, the blood could be sucked in by an attached rubber tube. Blood from the pipette was transferred direct to the thermocouples (kept in the moist chamber) almost immediately after removal because of its tendency to clot, especially in *Leander*. When drops of blood were mounted, the first drop from the pipette was not used, nor was the last, so as to reduce the errors due to evaporation to a minimum. No animal from which blood was removed once was ever again used except for the removal of urine.

The obtaining of samples of urine was considerably more difficult. Repeated trials showed that the following method gave the best results. The prawn, after the adhering water has been wiped off, is laid on its back on a glass plate under a binocular microscope; if this proves difficult it may be kept in position by a rubber band running round the plate. By careful manipulation, the opening of the antennary gland at the base of the antenna may now be located and its outside carefully wiped with strips of filter paper. The tip of a micropipette, with an attached rubber tube, is introduced into the pore of the antennary gland and the contents sucked into the pipette. In many experiments the hydrostatic pressure of the urine inside the bladder was sufficiently high to fill the pipette without much effort. While this procedure worked well with *Leander*, success was only partial with *Palaemonetes* owing to its small size. Some samples were obtained from larger individuals by the same method as the above. In others, only the contents of the nephroperitoneal sac were taken for estimation of osmotic pressure. In *Leander*, the contents of this sac (which is in direct communication with the bladder, Fig. 5, p. 332), as obtained by dissection, had nearly the same osmotic pressure as the urine collected at the base of the antenna (Table III). Applying this information to *Palaemonetes*, the contents of the nephroperitoneal sac may be expected to give an indication of the properties of the urine; the source of the samples is indicated separately in the tables.

The dry weight of specimens used for estimation of water content was taken after the animals had been crushed and kept in a hot-air oven at a temperature of 105° C. for 24–36 hr. which was about the time required to get constant weights. The weighings were to within 2·5 mg.

The freezing-point of the blood of *Leander serratus* was determined by a cryoscope of the Burian and Drucker model; the Beckmann thermometer was graduated to 0·02° C. and the readings taken were correct to about 0·01° C.

Osmotic Changes in the Blood of *PALAEMONETES*

Palaemonetes varians inhabits brackish waters in the vicinity of Plymouth and adjoining places; though it is common in waters of varying salinity on the English and Irish coasts, it is rarely, if ever, met with in the open sea. In northern and western Europe the species is known only from water with some admixture of salt, whereas in the Mediterranean it is a freshwater species; but even there it is not exclusively fluviatile as shown by Gourret's record of it from salt water in the south of France along with strictly marine species (Gurney, 1923). Physiological varieties of the species have been distinguished (vide Boas, 1898, for summary and literature) in accordance with the size of the egg and reproductive habits; the brackish-water form with smaller eggs and full complement of larval stages is called var. *microgenitor* (=var. *occidentalis*, Sollaud, 1923) and the freshwater variety with larger and fewer eggs and a reduced development is designated var. *macrogenitor* (=var. *lacustris* von Martens). Sollaud added a third variety *mesogenitor* from Tunisia, but this is probably a distinct species (Gurney, 1923). It may be pointed out that there is no important structural difference between these varieties. In a later paper, Sollaud (1932) has suggested that the four forms of *Palaemonetes* found around the Mediterranean which are usually considered as different varieties of *P. varians* are specifically distinct, though they form a natural group of their own ('clan') within the genus. Of these, *P. varians* has small eggs (microgenitor), *P. antennarius* and *P. mesopotamicus* have large eggs (macrogenitor), while *P. mesogenitor* shows the intermediate condition.

The locality from which specimens were obtained for this study is subject to wide fluctuations in salinity during the year. The Chelson Meadow consists of a large tract of unreclaimed land near the mouth of the Plym, from which it is separated by an embankment. Salt water enters during high tide and the excess of water drains off into the river through sluice gates. The animals that are commonly obtained with *Palaemonetes* in the ditches at Chelson Meadow are *Potamopyrgus*[1] *jenkinsi* and *Gammarus chevreuxii*, both of which are well known for their high degree of tolerance to salinity changes (Sexton & Matthews, 1913). During the course of this work the highest salinity observed was in the summer and autumn, and the lowest during the winter (Fig. 1).

The blood of *Palaemonetes* obtained from its natural habitats has an osmotic pressure varying between 2·0 and 2·4 % NaCl according to changes in the environment at different times of the year as shown in Fig. 1. The results plotted indicate an almost straight-line correlation except for the values obtained during February, which are comparatively higher, and of May (summer) which are lower. The blood is hypotonic in concentrations up to about 2·25 % when isotonicity is established. The blood is hypertonic in further dilutions of the environment, and the fall in osmotic pressure compared with that of the external medium is very limited, the lowest value being about 2·0 % NaCl. The blood of prawns that were living in the sea-water tanks for some months gave values of about 2·30 % NaCl. Thus in prawns taken from the natural habitats the maximum difference between the highest and lowest values for blood is only 0·40 % NaCl for a difference of nearly 3·20 % NaCl in the external medium, while under experimental conditions the corresponding value is nearly 0·50 % NaCl.

The osmotic changes in the blood of *Palaemonetes* when transferred from sea water to different experimental media are shown in Fig. 2A. The curve obtained for these values does not exactly correspond with the values for specimens from Chelson Meadow (Fig. 2). The difference seen is no doubt due to the slow and prolonged acclimatization which the experimental animals are denied. But this is not explained entirely on the basis of the time factor, since experimental animals have been observed to attain a more or less steady state after some time.

Palaemonetes is able to survive sudden transfer from sea water to water of very low salinity, i.e. about 0·5 % NaCl. The prawns remain active and healthy in spite of sudden changes in external medium and, by slow acclimatization, the animals could be brought down to water of as low an osmotic pressure as 0·01 % NaCl. If directly transferred to distilled water from sea water they survive for about 2–3 hr.; but in distilled water with a trace of sodium chloride (osmotic pressure 0·005–0·01 % NaCl) they live for 24–30 hr. Six specimens acclimatized to 0·45 % NaCl lived for 18–22 hr. in Plymouth tap water, and their survival period could be prolonged to several days by the addition of a small quantity of NaCl solution so as to raise the osmotic pressure of the medium to about 0·005–0·01 % NaCl.

Values of osmotic pressure of specimens transferred from sea water to

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[1] Also known under the names *Hydrobia* and *Paludestrina*.

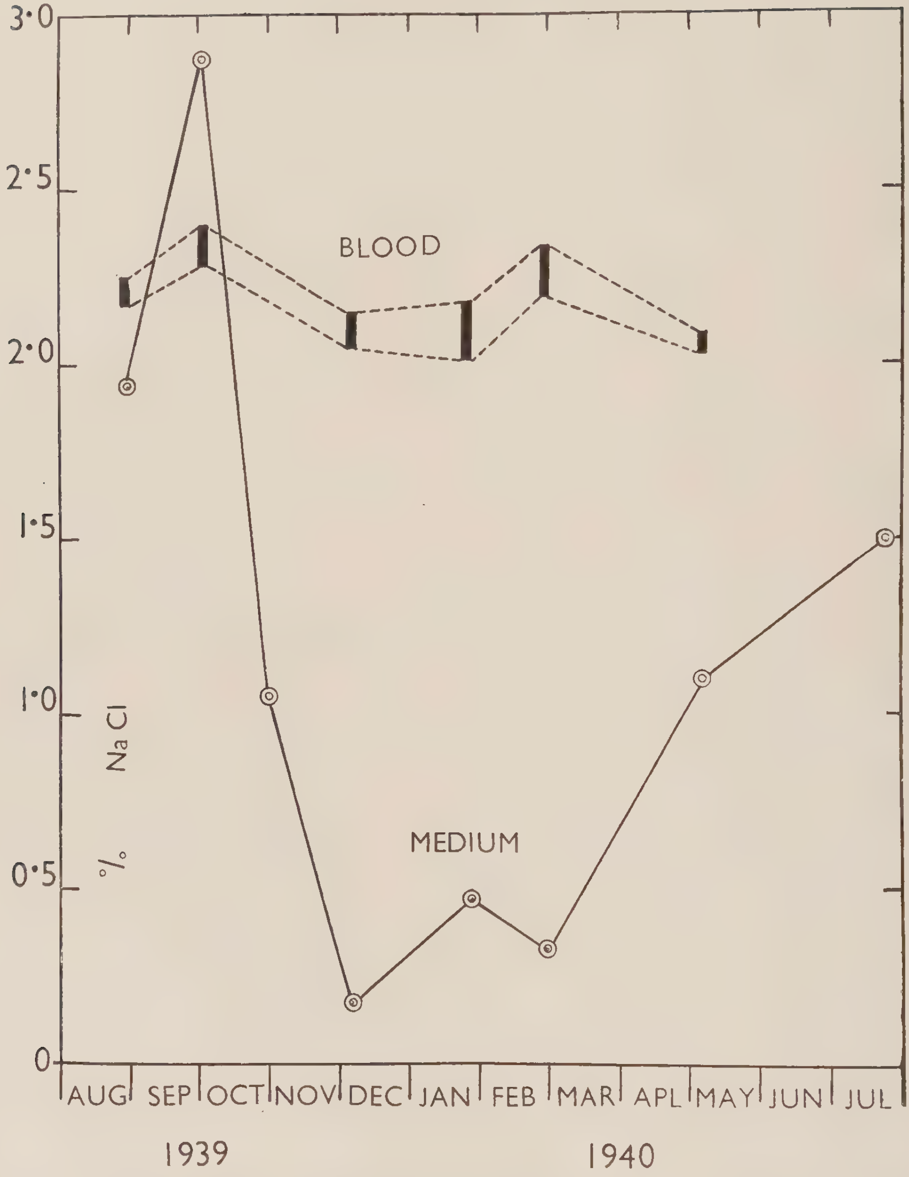

Fig. 1. Seasonal changes in the osmotic concentration of the blood of *Palaemonetes varians* in its natural habitat at Chelson Meadow. Ordinates represent osmotic pressure of blood (% NaCl) and the salinity of the external medium (expressed for convenience in % NaCl). The range of values for blood falls between the dotted lines.

water of low salinity and from water of low salinity to sea water are given in Figs. 3 and 4. In the first transfer (Fig. 3*a*) there is a steady fall in values from about 2·4 to nearly 2·0 % within the course of 8–10 hr., and an almost steady state is thereafter attained. The curve, however, is by no means smooth, which may be partly due to the differences in adaptive powers among the individuals taken for experiment. There is also an indication that the osmotic pressure fluctuates before a final steady state is reached, and this is also seen in animals transferred to sea water (Fig. 4*a*). In both experiments the animals show a direct and immediate response to changes in external osmotic pressure and the attainment of a steady state by later adjustment.

In 1925 Vialli made some measurements of the osmotic pressure of the freshwater variety of *Palaemonetes varians* from Italy by employing Monti's method. From thermoelectric readings of what he calls the coelomic cavity, he found that the prawns showed a Δ of $-0{\cdot}54°$ C. which is equivalent to about 0·92 % NaCl. Even the lowest value I have obtained in specimens acclimatized to nearly freshwater is considerably higher than Vialli's. Confirmation of his results would be of great interest, since it may indicate another physiological difference between the Mediterranean and Western European varieties.

By allowing the water in which they are kept to evaporate, or by the addition of small quantities of Tidmann's sea salt, specimens of *Palaemonetes* have been acclimatized to concentrations as high as 5·2 % NaCl. From sea water the prawns can survive direct transfer to concentrated sea water having a value of 5·0 % NaCl; but animals acclimatized to hypotonic media were unable to live long in water of 4·5 % NaCl after direct transfer.

Osmotic changes of *Palaemonetes* consequent upon direct transfer from sea water to concentrated sea water of 5·00 % NaCl are given in graphs *b* and *c*, Fig. 4. There is a steady rise in the values for blood to about 3·0 % within the first 4 hr., but afterwards there is great fluctuation in values. It is possible that some of the specimens which showed abnormally high values were those which in due course might have died owing to inefficient regulation, but at the time when estimations were made they were alive and active. The critical stage in adaptation is evidently after about 4–5 hr. when the osmotic pressure is already 3·0 % NaCl, since the high degree of scatter in the values is observed only after this period. It is noteworthy that prawns that lived in the medium for 3 days and had attained a steady state also show a value of nearly 3·0 %. In 4·78 % NaCl, the mean value for blood came down to about 2·6 % in a week's time (Table II).

The figures obtained when animals are subjected to sudden change of environment show definitely that there is an initial rise or fall in osmotic pressure which is considerably higher or lower than the final steady value. Thus the upset of osmotic equilibrium is not avoided by the animals by passive control, but adjustment is later effected by osmotic work done by them. The fluctuations in the values may largely be due to individual differences in adaptive capacity owing to differences in size and moult stage, but this cannot account for the rise and fall after 4 hr. in experiments with concentrated sea water. Possibly the entry of salts into the prawns may take place discontinuously, as for example when they occasionally drink water to make up for water loss, and this is partly responsible for the wide fluctuations.

It has already been mentioned that the osmotic pressure of the blood of animals taken from the natural habitat during February was slightly higher that one would expect from the curve. Similarly there is a steady fall from February to May in spite of the rise in salinity of the medium (Fig. 1). This would indicate a lower value in summer and a higher one in winter. A similar seasonal cycle has been observed by Widmann (1935) and Otto (1937) working with other Crustacea. According to their observations as well as mine, higher temperature seems to give lower values of osmotic pressure. The possible significance of this in certain problems of animal distribution has been discussed elsewhere (Panikkar, 1940*b*).

Palaemonetes is able to take in salt when in hypotonic media. Prawns collected from Chelson Meadow, found living in a medium of 0·175 % NaCl and having an average osmotic pressure of 2·108 % NaCl, were transferred to dilute sea water of 1·450 % NaCl. Osmotic changes cannot be expected since the new medium is also hypotonic to the blood, but after 48 hr. the average osmotic pressure of the prawns rose to 2·290 % NaCl. Judging from the analogy of freshwater and brackish-water organisms investigated by Krogh (1938) the gills would seem to be the primary site of absorption, though a small amount of salt may also be absorbed through the gut.

OSMOTIC CHANGES IN THE BLOOD OF *LEANDER*

Leander serratus

Leander serratus, the so-called 'common prawn', is a littoral marine species abundant in the southern parts of the North Sea and in the Mediterranean. Details of its distribution are given by Gurney (1923). Though it is able to withstand a certain amount of dilution of the environment, this prawn is essentially marine in its habits, but it is not uncommon to find it among sea weeds on the coastal regions where slight dilution of the sea water may take place from rain or river water. Unlike the closely related species *L. longirostris*, *L. serratus* does not seem to migrate much into rivers; nor does it show any preference for brackish water or estuarine habitats as do many other *Leander*.

The osmotic pressure of the blood of *L. serratus* when in sea water has a value of 2·6–2·9 % NaCl. The variations of osmotic pressure among individuals of the same batch is higher in this prawn than in *Palaemonetes*, and, as will be shown later, much of this variation may be expected on the basis of differences in moult stages. It is worth mentioning that my records show that, as in *Palaemonetes*, the blood shows a higher value in winter than in summer. The mean value for the blood of twelve prawns of about the same intermoult period, as judged by appearance, taken from sea water of 3·45 % NaCl in summer was 2·60 % NaCl, but the corresponding value for winter was 2·85 %. While there is no perceptible difference between males and females not in berry, the ovigerous females on the whole show a slightly higher value of 2·95 % (winter). I have not been able to find any definite correlation between size and osmotic pressure of blood among individuals averaging from 35 to 80 mm. in length, but the larger prawns of 96–100 mm. usually show higher values than smaller individuals of the same batch. In the absence of data on the moult stage of each prawn investigated, I am not inclined to emphasize these differences, especially when we consider that the larger prawns have a longer intermoult period.

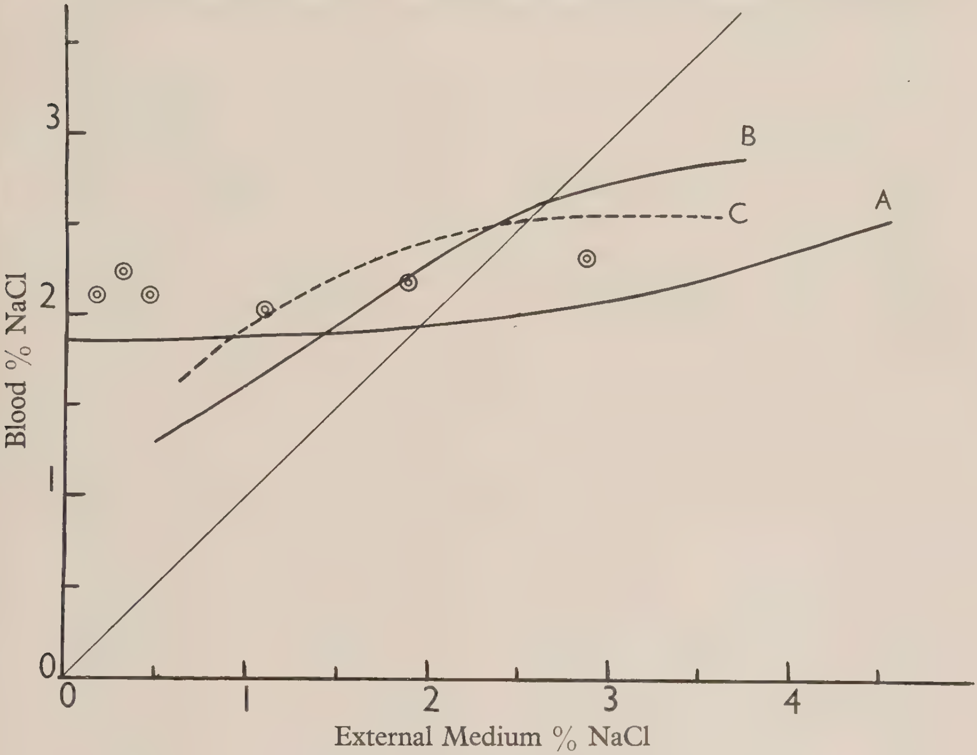

Fig. 2. Osmotic pressure (in % NaCl) of the blood of (A) *Palaemonetes varians*, (B) *Leander serratus* and (C) *Leander squilla* (dotted line) in different concentrations of the external medium measured under experimental conditions. Abscissae, external medium; ordinates, blood. Straight line indicates where points would fall if external and internal media were isotonic. Circles represent osmotic pressures (mean values only) of *Palaemonetes* from water of different salinities in its natural habitat.

Osmotic pressure of the blood of *Leander serratus* was also measured by the freezing-point method. Blood from about twenty-five specimens was collected for this purpose and allowed to stand for 6 hr., after which the clear bluish serum was separated and poured into the cryoscope. In two series of estimations with two sets of prawns taken from circulation in January 1941, Δ values of $-1{\cdot}70$ and $-1{\cdot}66$ °C. were obtained for the blood serum and of $-2{\cdot}06°$ C. for sea water. These values are in fairly close agreement with the results obtained by the vapour-pressure method.

Table III and the graph B in Fig. 2 show the osmotic relations of *L. serratus* in different dilutions of the environment. The prawn is homoiosmotic in normal sea water down to a dilution of sea water equivalent to 2·5 % NaCl, which is the approximate point of isotonicity. There is a steady decline in value after this, but even in lower dilutions the ability of the animal to maintain a hypertonic blood concentration is well marked. The curve has thus the essential features of the *Palaemonetes* curve, the differences observed being caused by the lower osmotic deficit when in sea water, and absence of efficient regulation in very dilute sea water. I have not been able to acclimatize *Leander serratus* to live in water below 0·6 % NaCl; the mortality was very high in experiments with water of 0·6–1·0 % NaCl, and no prawn which lived for a few days in these dilute media showed an osmotic pressure less than about 1·6 % NaCl.

Acclimatization experiments show that the degree of tolerance to changes in the external medium is very limited in *Leander* as compared with *Palaemonetes*, but certainly much better developed than in most marine invertebrates. It can survive direct transfer to media as low in concentration as 1·6 % NaCl. Even in lower dilutions the prawns live for 3–4 hr. after direct transfer and may recover if taken back to sea water before they have become comatose. In all these the size of the individual and the moult stage seem to have a close bearing on the ability to survive sudden salinity changes. Smaller juvenile prawns have a greater degree of tolerance than larger ones, and the ovigerous females have the minimum amount of tolerance to diluted media. Similarly, the freshly moulted individuals die in a few hours if transferred to sea water of even moderate dilutions, such as 2·0 % NaCl. The period of survival after such changes is longer the longer the time that has elapsed after moult.

Slow acclimatization to concentrated sea water is possible, the maximum upper limit being about 3·9 % NaCl, the osmotic pressure of blood rising in the meantime to 3·1 % NaCl. This value is not represented in curve B, Fig. 2, since the observations have not been extensive.

Osmotic changes of prawns when they are directly transferred to dilute sea water are shown in Fig. 3. As may be expected, the time taken to reach the lowest value varies according to the strength of the experimental medium and the sizes of individuals, the small prawns showing a higher rate of change. The dilution curve is steady until the final equalization, followed by a slight rise, the curve having the same shape as the one obtained for *Palaemonetes*.

Like *Palaemonetes*, *Leander serratus* is able to absorb salt from hypotonic surroundings. Prawns acclimatized to 50 % sea water (osmotic pressure

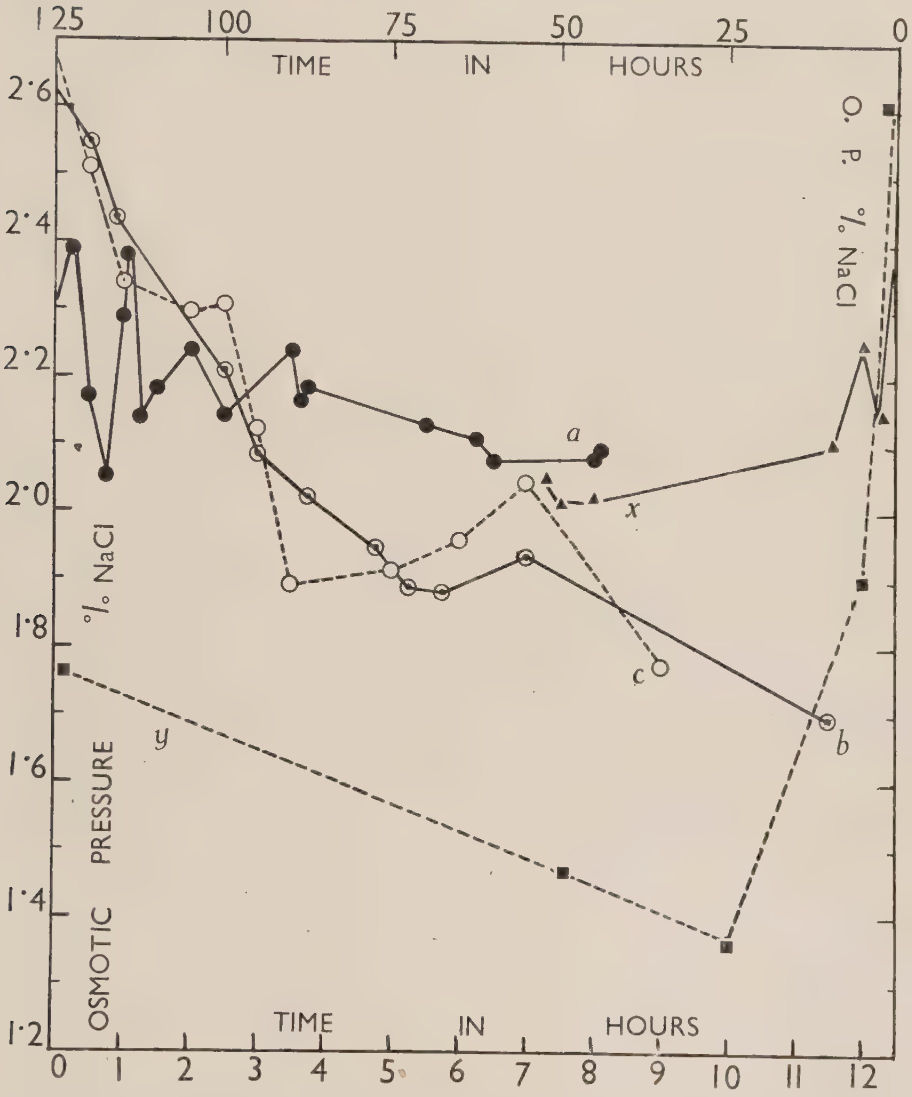

Fig. 3. Osmotic changes of *Palaemonetes varians* and *Leander serratus* when transferred directly from normal sea water to dilute sea water. Abscissae, time in hours; ordinates, osmotic pressure of blood in % NaCl. (*a*) *Palaemonetes* from sea water to dilute sea water of 0·565 % NaCl (9. xii. 39); (*b*) *Leander* from normal sea water to dilute sea water of 0·616 % NaCl (29. xi. 39); (*c*) *Leander* from sea water to dilute sea water of 0·65 % NaCl (dotted line, 6. xii. 39); (*x*) same experiment as (*a*) but continued for 54 hr.; (*y*) *Leander*, same experiment as (*b*) but carried on for 124 hr. (dotted line). Lower time scale for (*a*), (*b*) and (*c*); upper scale for (*x*) and (*y*).

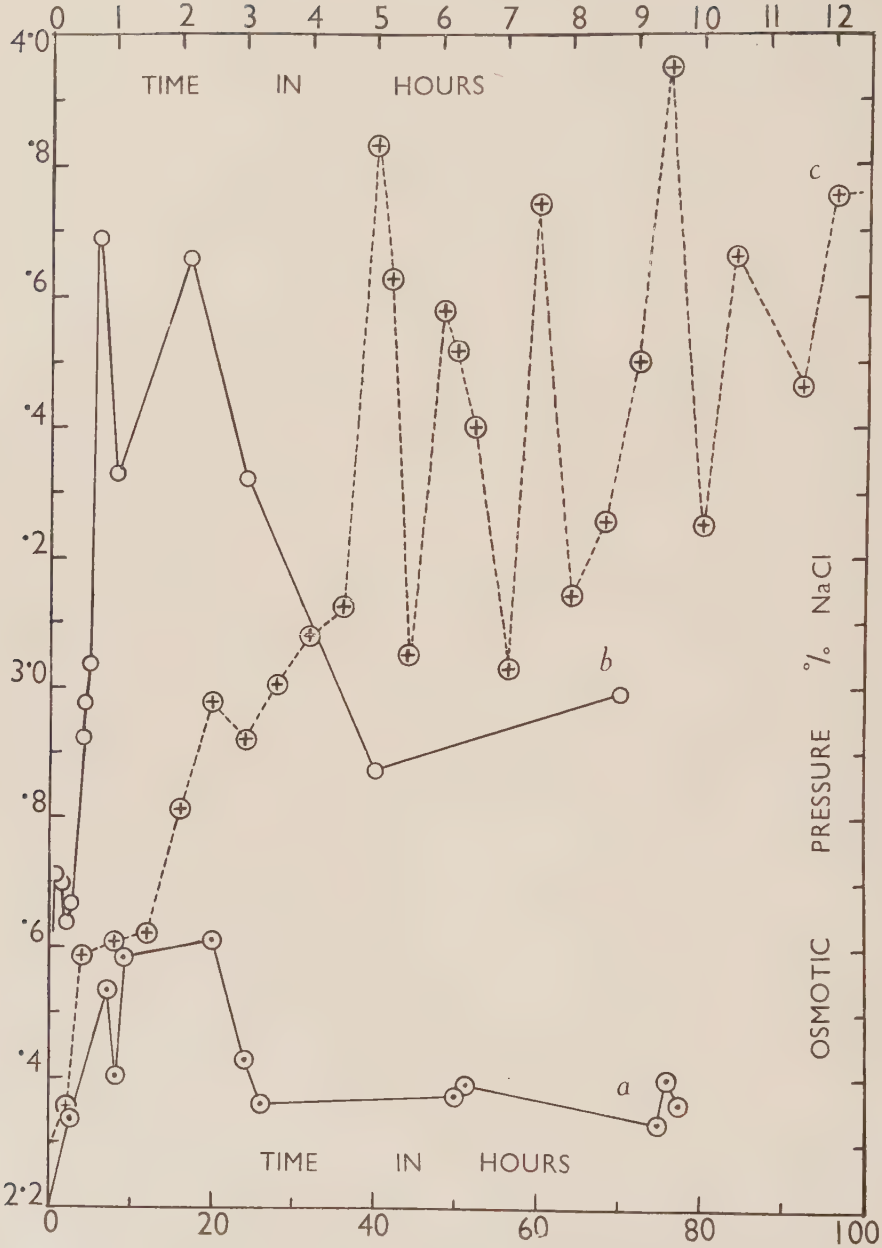

Fig. 4. Osmotic changes of *Palaemonetes varians* on direct transfer to concentrated media. Abscissae, time in hours; ordinates, osmotic pressure of blood in % NaCl. (*a*) From water of 0·325 % NaCl (natural habitat) to circulating sea water of 3·341 % NaCl (1. iii. 40); (*b*) from sea water of 3·4 % NaCl to concentrated sea water of 5·0 % NaCl (3. iii. 40); (*c*) from sea water of 3·4 % NaCl to 5·5 % NaCl (dotted line) the changes observed during the first 12 hr. Lower time scale for (*a*) and (*b*); upper for (*c*).

1·720 % NaCl) and having a mean osmotic pressure of 2·3 % NaCl showed an increase in value up to 2·5 % NaCl in 14 hr. when transferred to sea water of 2·2 % NaCl.

Leander squilla

Leander squilla (Linn.) is littoral in habits; the species enjoys a wide distribution, being common in the Baltic Sea, Scandinavian, Dutch, British and French coasts, the Mediterranean and the Black Sea (Gurney, 1923). It is frequently encountered in brackish water in the Black Sea and in pools in salt marshes on the English coasts along with *Palaemonetes varians*. In addition to occurring in the sea at Plymouth, the species has been recorded a few miles up the River Lynher, River Tamar and at Laira; in the spring of 1940 a few juvenile individuals were obtained along with *P. varians* at the Chelson Meadow. Gurney mentions that there can be no doubt that the whole larval life is spent in the sea. It was difficult to obtain a plentiful supply of this prawn during the period of study, and the observations on it are therefore not very extensive.

Fig. 2C gives the values of osmotic pressure of prawns from different salinities. In normal sea water the blood is equivalent to about 2·6 % NaCl; approximate isotonicity is established in a medium equivalent to about 2·5 % NaCl, and in lower dilutions the blood is hypertonic to an appreciable extent indicating effective regulation. The curve obtained when the results are plotted is very similar to that for *Leander serratus*. It is noteworthy, however, that the optimum value in sea water is slightly lower than in *L. serratus* (2·6 as against 2·8 %), and that the homoiosmotic behaviour is even better developed than in the former species. The osmotic adaptation is therefore intermediate between that of *Palaemonetes varians* and *Leander serratus*, and this is precisely what one would expect from its habits and distribution, and from the results obtained by Mathias (1938) and Pora (1938) in regard to its ability to withstand experimental changes in the external medium.

Osmotic Properties of the Urine of Palaemonids

Description of the Excretory Organs

The main excretory organs of prawns are the green glands or antennary glands which become functionally active as soon as the shell glands or maxillary glands disappear at the end of larval life. The green glands of the Palaemoninae have been described by Grobben (1880), Weldon (1889, 1891), Marchal (1892), Allen (1892*b*), Cuénot (1895) and Patwardhan (1937). The excretory pore, found at the base of the antennary peduncle in close proximity with the labrum, leads through the ureter into the bladder, to one side of which is attached the end-sac and the convoluted tubules of the labyrinth Fig. 5). The end-sac is a small, compact, specialized structure composed of an outer layer of connective tissue containing blood spaces and an inner layer, thrown into folds projecting into the lumen of the sac, which is lined with large excretory epithelial cells having conspicuous nuclei and granular cytoplasm. The excretory tubules forming the glandular plexus or the labyrinth anastomose freely with one another, but they communicate by means of a common opening with the end-sac and by several openings with the bladder. The walls of these tubules are formed by a single layer of excretory epithelial cells, while the intervening spaces between the tubules are filled with loose connective tissue and numerous blood lacunae. The bladder wall is extremely thin and lined with epithelial cells resembling those seen in the tubules.

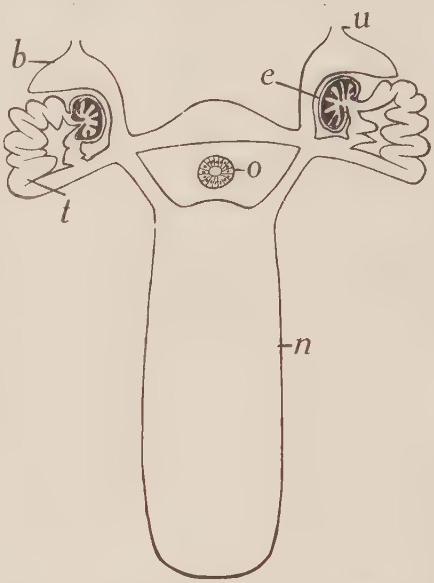

Fig. 5. Diagram of the palaemonid excretory system (after Weldon). *b.* bladder; *e.* end-sac; *n.* nephroperitoneal sac; *o.* oesophagus; *t.* tubule; and *u.* ureter.

Of special significance in the excretory system of the Palaemoninae is the large median renal or nephroperitoneal sac which is an unpaired structure lying in the cephalothorax dorsal to the cardiac stomach and ventral to the ophthalmic artery and the median dorsal sac. This communicates with the bladder by means of two lateral nephroperitoneal ducts which are also connected by a transverse commissure passing in front of the oesophagus. The renal sac is composed of a single layer of flattened excretory epithelium which both Weldon and Marchal found capable of absorbing indigocarmine and similar substances when they are injected into the blood of the living animal. From a study of the development of the excretory organs of *Palaemonetes varians*, Allen (1892*b*) demonstrated that the nephroperitoneal sac is formed by the fusion of the backward extensions of the two bladders. He also drew attention to the presence of a completely closed dorsal sac (the median dorsal blood sinus of Weldon) which lies upon the nephroperitoneal sac; it is probably coelomic in origin and persists in the adults of *Palaemon* (= *Leander*) and *Palaemonetes*. According to him it does not contain blood since blood corpuscles are never met with in the sacs of the larvae or of the adults; nor has any direct communication been observed between the dorsal sac and the blood sinuses of the body.

In Tables II and III are given the values of the osmotic pressure of the urine of *Palaemonetes* and *Leander*. In general the osmotic pressure of the urine is closely similar to that of the blood, though slight variations on either side are observed, as was also noted by Picken (1936) and Nagel (1934) in

Carcinus. The results also indicate that the general rule of isotonicity with blood is not affected by the differences in the nature of the medium, whether hypotonic or hypertonic. In *Palaemonetes* transferred to fresh water with a little NaCl, the urine was found to be slightly hypotonic to the blood, which also had a comparatively low value. But even here the extent of hypotonicity is so small that it may not be considered far outside the range of experimental variation, and further, the steady state had not been attained as evidenced by the low value for blood.

TABLE II. OSMOTIC PROPERTIES OF THE BLOOD AND URINE OF *PALAEMONETES VARIANS* IN DIFFERENT MEDIA. MARCH 1940

			Osmotic pressure, % NaCl		
No.	Length mm.	Time	Blood	Urine	Medium
1	28	1 week	2·606	—	4·780
2	29	1 week	2·583	—	4·780
3	27	1 week	2·692	—	4·780
4	27	10 days	2·745	2·615	4·225
5	27	10 days	2·546	2·500	4·225
6	27	15 days	2·315	2·318	3·410
7	28	1 month	2·224	2·290	3·246
8	29	Nat. habitat	—	2·158	0·468
9	28	Nat. habitat	—	1·952	0·468
10	28	Nat. habitat	2·013	—	0·468
11	27	Nat. habitat	2·188	—	0·468
12	33	15 days	1·907	1·918*	0·446
13	32	15 days	1·935	1·930*	0·446
14	32	15 days	1·945	1·969*	0·446
15	22	44 hr.	1·106	0·984	0·017
16	20	45 hr.	1·165	1·145*	0·017
17	28	Gradually acclimatized	1·892	—	0·010

* Sample from nephroperitoneal sac.
No. 15 showed signs of distress; no. 16 was a healthy specimen.

TABLE III. OSMOTIC PROPERTIES OF THE BLOOD AND URINE OF *LEANDER SERRATUS*. FEBRUARY 1940

Length mm.	Time	O.P. medium % NaCl	O.P. blood % NaCl	O.P. urine % NaCl
90 (ov. female)	2 months	3·246	2·960	2·991
94	2 months	3·246	3·046	2·955
90 (ov. female)	2 months	3·410	—	3·000
95 (ov. female)	2 months	3·400	2·934	2·958
55	1 month	3·210	2·608	2·598*
57	11 days	2·873	2·547	2·536*
57	11 days	1·646	2·180	2·190
62	11 days	2·873	2·500	—
75	½ hr.	Tap water	2·559	1·420 (?)
80	1 hr.	—	2·479	2·477

* Sample from nephroperitoneal sac. All others collected from the opening of the antennary glands.

The contents of the different parts of the excretory system of three specimens of *Leander* were removed separately and their osmotic pressures estimated to find if significant differences could be discovered (Table IV). The method adopted was to dissect the prawns under the binocular microscope, after blood had been collected from the heart and urine from the opening of the antennary gland. The contents of the nephroperitoneal sac and the dorsal sac were collected in separate micropipettes. While reasonably pure samples could be obtained from the nephroperitoneal sac, the possibility of contamination with blood could not be completely ruled out in collecting from the dorsal sac, though every possible care was taken to make sure that the sample was pure. The values obtained are nearly identical with the blood except for slight variations which are well within the limits of experimental error. Similar analysis could not be carried out with *Palaemonetes* owing to its smaller size. The fact that there is isotonicity of urine with blood in *Palaemonetes* acclimatized to water of extremely low salinity, and the almost similar values obtained for the contents of the different parts of the green gland of *Leander*, would point to the conclusion that there is no mechanism for the production of urine hypotonic to blood in either of these prawns.

TABLE IV. ANALYSES OF SAMPLES FROM DIFFERENT PARTS OF THE EXCRETORY SYSTEM OF *LEANDER SERRATUS*. OSMOTIC PRESSURE IN % NaCl. MARCH 1940

No.	Medium	Blood	Urine from bladder	Nephro-peritoneal sac	Dorsal sac
1	3·400	2·781	2·792	2·691	2·701
2	3·280	2·760	2·698	2·720	2·770
3	3·280	2·810	2·779	2·791	2·828

It may be mentioned here that, in addition to the antennal glands, the gills may be expected to have a marked excretory function as shown by the work of Mollitor (1937) on the crab *Eriocheir*.

OSMOTIC CHANGES IN RELATION TO MOULTING

Interesting osmotic changes in accordance with the moult cycle were discovered by Baumberger & Olmsted (1928) in the crab *Pachygrapsus crassipes*. In sea water having a freezing-point depression of $-1·975°$ C. the tissue fluids of hard crabs had a Δ of 1·327°, of pillans[1] 1·893°, of hard crabs about to moult 2·601°, and of newly moulted crabs 2·193°. Thus the crabs are normally hypotonic to the extent of $-0·648°$ C. when they are far from moult, but in most other stages of the moult cycle the blood is hypertonic to the external medium. It has been concluded that there is a large increase in blood concentration of crabs just before moult and that a considerable amount of water is absorbed immediately after moult, which accounts for the reduction of osmotic pressure to normal. These changes have been confirmed in *Callinectes sapidus* (Baumberger & Dill, 1928) and in *Carcinus maenas* as regards water content (Robertson, 1937). There is no reference in the literature to osmotic changes in Macrura as influenced by the moult. In *Homarus* it has recently been found that osmotic equilibrium is established soon after moulting (Lowndes & Panikkar, 1941). The following observations are of interest, since in no stage of the moult cycle is *Leander* or *Palaemonetes* hypertonic or even isotonic to the environment, thus offering a striking contrast to the condition reported for *Pachygrapsus crassipes*.

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[1] A stage intervening between 'hard' and 'about to moult' crabs, characterized by cracks on the carapace. Also called 'peelers'.

In prawns it is by no means easy to judge the moult stages by appearance; nor is it possible to classify them into different moult groups as was done by Baumberger and Olmsted in *Pachygrapsus*. The prawns usually moult during the night and the moulting process itself is rapid; it was thus not possible to obtain a large number in which the time which had elapsed since the moult had been definitely ascertained. The procedure adopted was to keep isolated individuals under observation. The moulting has been described by Gurney (1923) in *Leander longirostris* and Nouvel (1933) in *L. serratus*, and I have little to add to these accounts. According to Nouvel the intermoult period varies from 10 to 20 days—the interval being longest in large prawns. The intermoult period was much longer in the batch of prawns observed by me; in young prawns it varied from 15 to 25 days, while the larger ones took about 30–35 days. In addition I have had under observation large prawns that had not moulted for 40–50 days.

Table V. Osmotic Changes of *Leander serratus* in Relation to Moult Cycle. Temperature 6–12° C.

Date 1940	Length of prawn mm.	Time after moult	Osmotic pressure blood, % NaCl	Osmotic pressure medium, % NaCl
21. iii	60	½ hr.	3·030	3·400
22. iii	51	1 hr.	2·984	3·400
15. ii	41	12 hr.	2·870	3·400
12. iii	90	15 hr.	2·815	3·342
14. ii	51	24 hr.	2·820	3·332
14. ii	54	24 hr.	2·932	3·210
23. iii	60	25 hr.	2·730	3·400
23. iii	95	10 days	2·635	3·400
28. ii	45	24 days	2·688	3·341
28. ii	102	38 days (ov. female)	2·913	3·341
14. ii	100	Far from moult	2·990	3·332
18. i	94	Far from moult. New moult imminent as judged by appearance	3·046	3·246

The values given in Table V show that the prawns have a higher osmotic pressure than normal immediately after moult, but the concentration comes down to normal in about 10 days' time. Expressed in terms of difference

TABLE VI. WATER CONTENT OF *LEANDER SERRATUS*

Prawns from sea-water circulation except 1 and 2

No.	Moult stage	Length mm.	Gross weight g.	Dry wt. g.	% water	% water, cast included
1	15 min. after moult in Ca free sea water	55	1·416	0·307	—	78·32
2	½ hr. after moult in art. sea water with calcium	67	2·717	0·564	80·28	79·28
3	½ hr. after moult	60	1·180	0·242	79·5	78·17
		Moult alone	0·240	0·068		
4	2 hr. after moult	59	1·120	0·230	79·46	77·98
5	3 hr. after moult	60	1·348	0·278	79·38	—
6	1 day after moult	60	1·240	0·268	78·4	—
7	4 days after moult	55	1·166	0·287	75·39	74·57
		Moult alone	0·234	0·069		
8	9 days after moult	95	5·850	1·370	76·6	—
9	30 days after moult	63	1·630	0·448	72·75	—

%	Before moult	After moult
Water	72·7	79·23
Solid	27·3	20·77

Amount of water in a prawn of dry weight 2·73 g. after moult $= \frac{7·923 \times 2·73}{2·077} = 10·414$ g.

Therefore the amount of water absorbed $= 10·414 - 7·270 = 3·144$ g.

Thus a prawn of fresh weight 1 g. absorbs 0·314 g. of water during moult.

between internal and external media the osmotic deficit is least in newly moulted examples and the deficit grows as days elapse after the moult. I have not been able to establish any remarkable rise in osmotic pressure immediately before moult, but it may be that I have not been able to pick prawns that were actually in the throes of moulting. In two prawns far from moult a slight rise in osmotic pressure was observed, and this is probably an indication of the activity prior to moulting. While a slight rise from 2·6 to about 3·0 % NaCl is indicated, there is no evidence that the blood attains hypertonicity with the external medium, nor even isotonicity, for the blood of a prawn only 10 min. after the moult gave a value of 3·00 % with an appreciable osmotic deficit of 0·37 % NaCl.[1] The general trend of osmotic changes in *L. serratus* during the moult cycle is similar to that of *Pachygrapsus*, with the difference that the range of variation is so limited that the hypotonicity of the blood is maintained in all stages of the moult cycle.

The exact mechanism whereby the increase in concentration is achieved prior to moult has not been satisfactorily explained in any crustacean. It would at least be partially explained by the conversion of accumulated glycogen into sugar as shown by Drilhon (1933) for *Maia*, though it should be admitted that the change in the sugar content of the blood observed in *Callinectes* (Baumberger & Dill, 1928) is insufficient to explain the whole osmotic rise. That the absorption of water is mainly responsible for bringing down the value to normal could be demonstrated by calculations based on

[1] This value is not included in Table V, only a single estimation having been made.

changes in water content. In prawns immediately after moult, the water content is about 79 %; a slightly lower value of 78 % is obtained if the cast skin is also included in the determination of gross and dry weights. On the other hand, prawns several days after moult have a water content of only about 72 % (Table VI).

It is noteworthy in this connexion that Robertson (1937), working on *Carcinus*, found that a crab having a fresh weight of 50 g. would absorb 35 g. of water during moult. The corresponding figures for *Maia squinado* (Drach, 1936), *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* (Olmsted & Baumberger, 1923) are 58, 17 and 22 g. respectively, while the corresponding value for *Leander serratus* is only 15·7 g. Lowndes & Panikkar (1941) have found that *Homarus* of fresh weight 100 g. absorbs about 47 g. of water within less than 2 days after moulting. The difference between the amount of water absorbed by the stenohaline *Maia* and euryhaline *Carcinus*, and the much lower value for *Pachygrapsus crassipes* and *Leander serratus* would seem to be closely related to the differences in the permeability of the integument. We should expect a low permeability for animals that can maintain hypotonicity, and if the amount of water absorbed and the consequent osmotic changes taking place form an index of permeability, *L. serratus* would seem to be the least permeable of all the above-mentioned crustaceans.

TABLE VII. OSMOTIC CHANGES OF *PALAEMONETES VARIANS*

Date 1940	Length mm.	Time after moult	Osmotic pressure	
			Blood	Medium
11. iii	40	6 hr. after moult	2·410	3·603
	40	Unmoulted for 32 days. Same lot as above	2·382	3·603
10. iii	27	1 hr. after moult	1·929	1·000
	30	Unmoulted for a very long time	2·034	1·000

I have not been able to make many observations on moulting in *Palaemonetes*, but Table VII gives values of osmotic pressure observed immediately after moult in two prawns that have been in experimental media. The figures, as compared with the normal, indicate that a slight rise may be expected, but distinctly smaller than in *Leander*; the amount of water absorbed after moult may be correspondingly low.

CHANGE IN WEIGHT OF PRAWNS IN DILUTED AND CONCENTRATED MEDIA

When a typical marine invertebrate is transferred to dilute sea water osmotic movements of water and salts take place resulting in a fall in the osmotic pressure of the internal medium. Direct determinations of osmotic pressure have shown that such a fall takes place in both *Palaemonetes* and *Leander*. If the dilution of blood is due to leakage of salts alone, there would be no appreciable difference in weight; but if it is caused by water entering the animal the weight of the animal should show a transitory increase. The changes in weight of *L. serratus* after transferring from sea water to 50 % sea water are given in Figs. 6 and 7 which are self-explanatory. An increase in weight is observed in all the three instances, but the time taken to reach the maximum is different in different individuals owing probably to differences in size and in the moult cycle which has a considerable influence on permeability. An increase of 6–9 % of original weight is noticed, but an increase above 6·0 % would seem to be fatal, as shown by the fact that the individual that survived for a long time after direct transfer showed only a maximum of 6·0 % and the values began to fall after about 25 hr. About 4 days were necessary for the prawn to show a constant weight, and this was about 2 % higher than the original weight. Experiments with individual specimens of *Palaemonetes* were not possible owing to their smaller size and the consequent tendency for the errors to be magnified, but a definite increase in weight has been observed in this species as well (Fig. 6*a*). Ten *Palaemonetes* having an initial weight of 1·86 g.

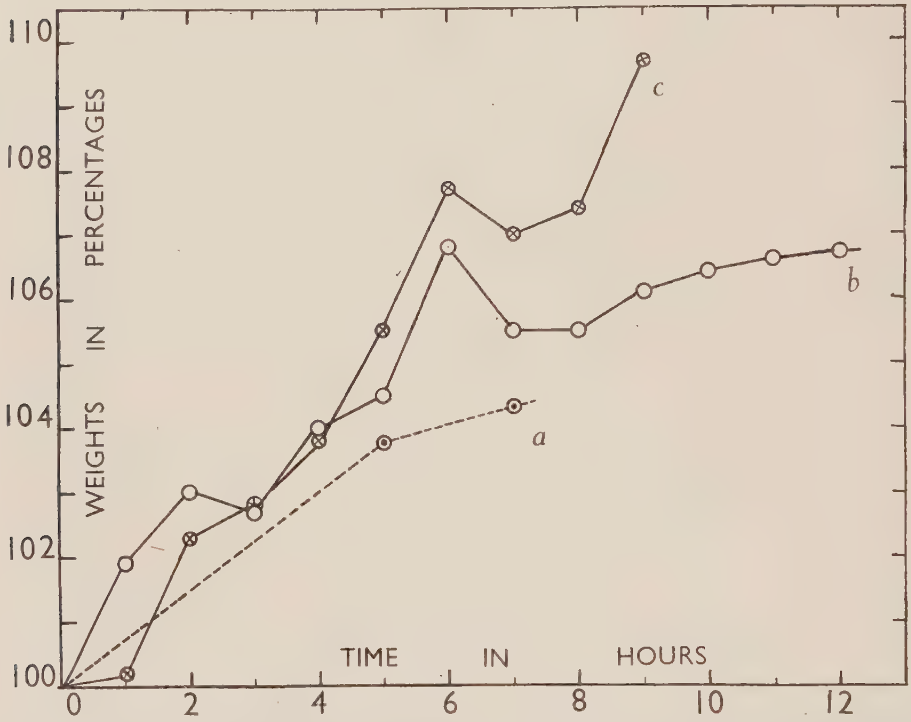

Fig. 6. Increase in weight of prawns in diluted media. Abscissae, time in hours; ordinates % of original weight. (*a*) *Palaemonetes* (dotted line), 10 specimens weighted collectively after transfer from normal sea water to 33 % sea water. (*b*) and (*c*) *Leander serratus* from sea water to 50 % sea water, single individuals.

in sea water, where they had been living for months, showed an increase in weight of 0·07 g., i.e. nearly 3·8 %, after remaining for 5 hr. in 33 % sea water. The increase was well over 4 % after 7 hr.

Similar increase in weight has been noticed in many other marine crustaceans, such as *Cancer pagurus* (Schlieper, 1929), *Portunus puber* and *P. depurator* (Hukuda, 1932); but Bethe (1930) failed to notice any appreciable increase in *Carcinus maenas*. In the mangrove crab, *Heloecius cordiformis*, Dakin & Edmonds (1931) failed to observe any change in weight corresponding to changes in the medium. In the brackish-water *Gammarus duebeni* and the freshwater *Gammarus pulex*, Beadle & Cragg (1940*a*) observed no appreciable change in weight; they conclude that in both these species changes in the osmotic pressure of the blood are due to salt and not to water movements. The changes in weight observed in *Leander* and *Palaemonetes* indicate definitely that the gills of both the species are permeable to water and that the dilution of the blood consequent on sudden transfer to dilute media is mainly brought about by the water that enters. A few hours after transfer a slight swelling and a high hydrostatic pressure of blood may be noticed in *Leander*. In the crabs studied by Hukuda a change of only about 4 % of weight was noticed and, by comparing with calculated figures assuming semipermeability,

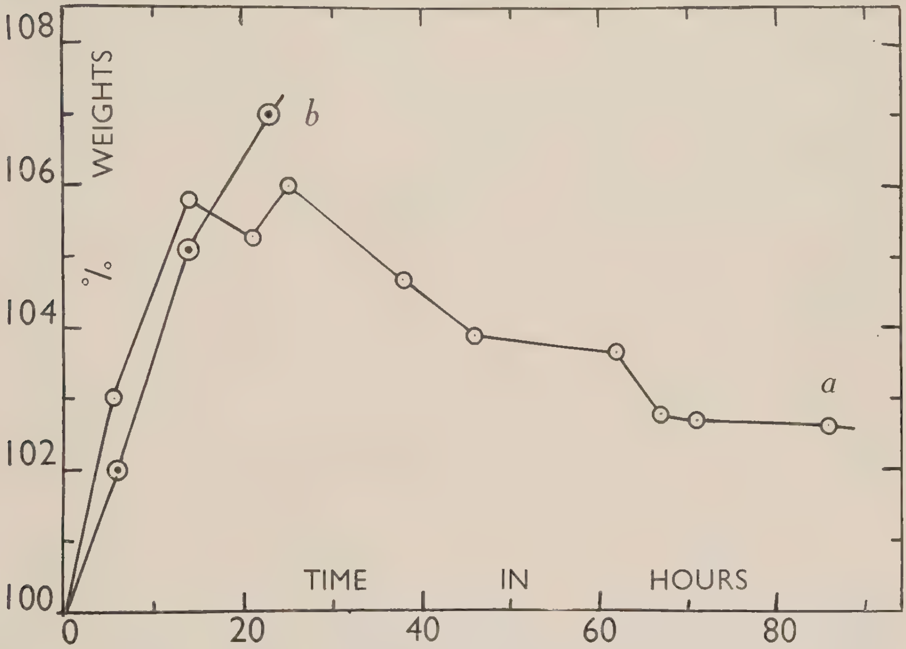

Fig. 7. Increase in weight of *Leander serratus* taken from sea water to 50 % sea water. (*a*) Specimen far from moult, which survived the experiment. (*b*) Prawn 2 days after moult which died in 24 hr.

the weight increase due to entry of water was shown to be insufficient to account for the fall in osmotic pressure of blood; he was therefore able to demonstrate the passage of an appreciable amount of salt to the exterior. Similar calculation with *Leander* would show that the amount of water that enters must be to a large extent responsible for the dilution of the blood, since the extent of dilution taking place is small owing to the low normal value for blood. The escape of salts to the exterior would seem to be inconsiderable at least in moderately dilute sea water, but it certainly takes place when the medium is very dilute.

When prawns are transferred from dilute to concentrated sea water there is a corresponding fall in weight, giving clear evidence of the escape of water to the exterior. The results obtained in experiments with *Palaemonetes varians* and *Leander serratus* are given in Fig. 8.

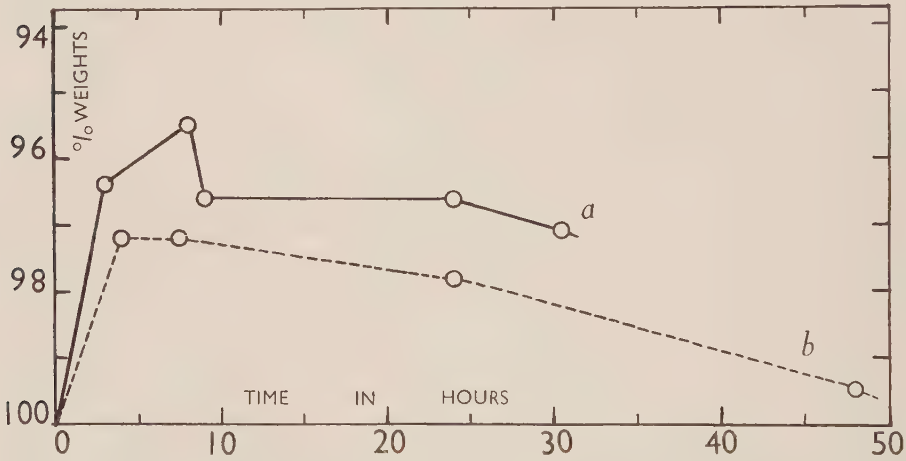

Fig. 8. Decrease in weight of *Palaemonetes* and *Leander* when taken to concentrated media. (*a*) *Leander serratus* acclimatized to 50 % sea water transferred to normal sea water. (*b*) *P. varians* acclimatized to 10 % sea water transferred to normal sea water; 10 specimens used collectively. (20. v. 40.)

PERMEABILITY OF THE GILLS

In Crustacea the integument has a very low permeability, being heavily impregnated with chitin and protected by cuticle. The only possible surface through which water and salts may freely diffuse into the body of the animal is that afforded by the gills. The following experiments were performed to ascertain the extent to which the gills of *Leander* and *Palaemonetes* are permeable.

A strong solution of methylene blue was dialysed into sea water kept in a jar until the sea water was rich blue and opaque. Specimens of *Leander serratus*, *Crangon vulgaris* and *Carcinus maenas* were transferred to the jar from the sea-water tanks. The *Leander* and *Crangon* chosen were approximately of the same size. The animals were removed at the end of half an hour, dissected, and the gills examined under the microscope. Of the preparations from the three species, the presence of methylene blue in the cells of the gill plates could easily be observed in *Crangon* and *Carcinus*, whereas no trace of the dye could be detected in the cells of *Leander serratus*. When the animals were left for about an hour, the gills of *Crangon* and *Carcinus* assumed a rich blue colour distinguishable even with the naked eye. In contrast to this, *Leander*, even if left for hours, failed to absorb the dye; the gills became faint blue after several hours, but this was owing to the presence of the dye between the gills and not in the gill cells proper.

The same experiment was performed with a methylene blue solution made in distilled water to which a small quantity of sea water was later added. This was necessary to prevent the early death of the experimental animals which would otherwise result. The osmotic pressure of this very dilute solution was 0·06 % NaCl. The results were similar to those of the previous experiment. The gills of *Crangon* and *Carcinus* took the dye in a very short time, the former being the first to show its presence. Even after 7 hr. of immersion the gills of *Leander* were colourless and the dye did not penetrate even 1 hr. after the death of the experimental animal.

Experiments with *Palaemonetes varians* gave the same results. The behaviour of the animal was also observed with methylene blue dissolved in concentrated sea water. The use of methylene blue is open to criticism owing to the fact that it may have been reduced after penetration (vide Gray, 1931). If an acidic dye like vital red is substituted, the dye has been observed to penetrate in *Leander* after prolonged immersion, while about half an hour is enough for *Crangon* and *Carcinus*. In regard to *Palaemonetes* the amount of vital red that penetrates (if any does) is extremely small, as judged by the examination of the gills of prawns appropriately treated. With a quick penetrating dye like neutral red, the gills of living *Crangon* are stained in a few minutes, while even after half an hour only a slight trace of the dye could be seen in *Leander*. These observations, though of a preliminary character, demonstrate that in *Leander* and *Palaemonetes* the permeability of the gills is very low as compared with the marine *Crangon* and the euryhaline *Carcinus*, or at any rate that the membrane properties of the palaemonid gills are different from that of the others.

Water-drinking Habits of Prawns

If a prawn is losing water by diffusion owing to the higher osmotic pressure of the external medium, one of the ways of making up for the water loss is for it to drink water. The technique employed to verify this water-drinking habit was essentially the same as that of Homer Smith (1930) who used phenol red as a suitable indicator. In addition to this dye, I have also employed vital red and brom-cresol green. Concentrated solutions of these dyes were added in small quantities to jars containing prawns in experimental media, and the animals were examined after suitable intervals to find if the dye could be detected in the alimentary canal. Unlike the teleosts, the dyes seldom reach the hind-gut or even the posterior half of the mid-gut, since the absorption seems to take place in the hepatopancreas and part of the fore-gut. One difficulty encountered in this connexion was the presence of pigmented cells in the hepatopancreas which are themselves subject to colour variations. By careful examination of teased tissues under the microscope it was, however, possible to distinguish the foreign dye as distinct from the natural pigmentation.

Both *Palaemonetes varians* and *Leander serratus* have been observed to drink the external medium, especially when the latter happens to be sea water or concentrated sea water. It must be mentioned, however, that even when the external medium is hypotonic they have been observed to take in water as judged by the presence of dyes inside them. The results obtained have been on the whole erratic. While (after proper treatment for *p*H differences) the dye became detectable in the hepatopancreas of some specimens in a few hours, in others I have not been able to observe the dyes even after 14 hr. In one set of experiments when solutions of the same concentration were used as the external media (sea water) for both the species, it was found that the dye was seen in *Palaemonetes* in 1 day while it took 4 days in *Leander*. Experiments in sealing the mouth and preventing the entry of sea water were not successful, since the oral appendages are always at work and remove any foreign body near the mouth.

The fact that water drinking may occur in both hyper- and hypotonic media and the wide divergence in results obtained with different individuals in the same medium seem to indicate that it is not an essential part of the osmoregulatory mechanism. It is probable that water gets into the alimentary canal when the prawns feed or when they attempt to search for food. When directly transferred to very concentrated media there is some evidence from osmotic changes to show that salt water is taken in from the surroundings (see p. 326).

Discussion

Mechanism of Osmoregulation

If an animal is able to maintain a lower osmotic pressure of the blood than the sea water in which it lives, it must either be completely impermeable to water and salts or it must be doing continuous osmotic work. Complete impermeability is not practicable in marine animals that depend upon the external medium for their essential supply of oxygen which has to diffuse through the gills. If, on the other hand, salts from the external medium penetrate the integument and water escapes in accordance with the laws of osmosis, the animal has first of all to make up for the loss of water it sustains and, secondly, it must get rid of salts to maintain the osmotic deficiency. The mechanisms possible are: (1) by producing a concentrated and markedly hypertonic urine, the animal can achieve elimination of excess of salts, and this may be further elaborated by the development of a special water re-absorbing mechanism; (2) it may drink sea water from time to time and thus make up for the loss of water and simultaneously excrete the excess of salt from the body, not necessarily through the agency of the kidney; or (3) the animal may absorb water from the surrounding sea water but not salt. The capacity to produce hypertonic urine has so far been observed only in vertebrates which have become terrestrial in habits, under conditions where the problem of conserving water is of paramount importance; none of the primarily aquatic organisms is known to possess it. In the marine teleosts, which have an osmotic pressure about one-third that of sea water, it has been demonstrated by Homer Smith (1930) and Keys (1931, 1933) that regulation is effected by the drinking of sea water and excretion of salts by the gills. We have seen that in *Leander serratus* and *Palaemonetes varians* the urine is isotonic with the blood whether the external medium is of extremely low or high salinity. The fact that the animals drink sea water and do this much more when in hypertonic media might suggest at once that the regulatory mechanism of palaemonid prawns is probably similar to that of marine teleosts. But this explanation alone would be insufficient to account for the osmotic independence of these prawns to wide variations in environment, and the ability, at least in *Palaemonetes*, to live in abnormally high concentrations of sea water which would be lethal to most marine animals—an ability comparable only with the classical instance of the brine shrimp *Artemia*. It seems likely that active water transport from outside to inside takes place in order to maintain hypotonicity in sea water in the same way as active salt absorption takes place in dilute sea water.

The total osmotic pressure curves of *Palaemonetes* and *Leander* indicate beyond doubt the homoiosmotic behaviour which they exhibit when their media are changed; this power is markedly developed in the former and partially lost in the latter. Within a range of nearly 5·0 % NaCl in its external medium the blood of *Palaemonetes* is affected only to the extent of about 0·8–1·0 % NaCl. The range of variation is much greater in *Leander*, but even here the blood is little affected by the dilution of the environment up to about 2·5 % NaCl; and it is probably reasonable to assume that under no circumstances is *L. serratus* subjected to changes in environment below this range under natural conditions. *Palaemonetes*, on the other hand, inhabits regions subject to wide fluctuations in salinity, not only seasonal but also tidal, the ditches in which it lives being liable to evaporation and to inundation by sea or river water. *Leander squilla* shows an intermediate condition which is what one would expect from its habits. But while the difference in homoiosmotic behaviour is consistent with the habits of these prawns, it does not enable us to judge how a new physiological ability has been acquired in one genus that is anatomically so similar to another, or how the palaemonid prawns have acquired an osmotic behaviour so different from other marine prawns.

When *Palaemonetes* is in water of extremely low salinity it has an osmotic pressure equivalent to about 2·0 % NaCl under natural conditions and about 1·8 % NaCl under experimental conditions. If the curve be projected to the freshwater limit the value cannot be below 1·7 %, and under conditions in which the species has become naturally acclimatized to fresh water[1] it is likely that the value is even slightly higher. This is no doubt much higher than the values of the osmotic pressure of most other freshwater Crustacea. The crayfishes *Potamobius* and *Cambarus* have blood with freezing-point depressions of about −0·8° C. (about 1·37 % NaCl) and −0·65° C. (about 1·11 % NaCl) respectively (Duval, 1925; Schlieper, 1935; Lienemann, 1938), and freshwater insects such as the mosquito (*Aedes argenteus*, Wigglesworth, 1933) and chironomid larvae have lower values (Harnisch, 1934; Koch, 1938), while freshwater branchiopods (*Daphnia*, Fritzsche, 1917; *Chirocephalus*, Panikkar, 1941) have osmotic pressures lower than in any other crustacean. In these freshwater forms there is a regular osmotic stream of water entering the body through the gills and escaping through the excretory system. In *Potamobius*, Peters (1935) has conclusively shown that the danger of loss of salts through the discharge of large amounts of urine is overcome by the salt reabsorbing mechanism resident in the nephridial canals, which makes the final urine extremely hypotonic to the blood. In the same way as freshwater organisms depend upon an osmotic stream of water for copious urine production, the marine teleosts depend upon the continuous drinking of sea water to cope with the elimination of salts through the gills. If the question of osmotic regulation is entirely dependent upon the continuous entry of water or salts through a semi-permeable membrane and the consequent elimination of water or excretion of salts, it would hardly be possible for animals to survive sudden changes in environment, since equally sudden reversal of physiological processes could seldom happen. It becomes evident, therefore, that the ability to control the exit and entrance of substances in the body of aquatic animals is an integral part of the regulatory mechanism. It may be achieved by different methods, as by the development of a keratinous waterproof coat in the majority of teleosts, the secretion of mucus in the eels (Duval, 1925; Keys, 1933), or by the development of an almost impermeable cuticle in arthropods. The factor of salt retention, recently emphasized by Beadle & Cragg (1940*b*), would seem to be the result of the low permeability of the integument.

Permeability of the Integument: Water and Ion Transport

It is well known that the crustacean integument has a low degree of permeability except in the region of the gills, where exchange of substances can take place in both directions. Different degrees of permeability have been noted by previous authors. In most marine Decapoda, Bethe (1929, 1930) has shown an almost indiscriminate permeability (of the gills) to water and ions; but these are forms in which the external and internal media are iso-osmotic. The ionic composition of the blood of these animals and that of sea water need not, however, be the same as shown by Bethe & Berger (1931) and by the recent analyses of Robertson (1939), but, in any case, a fairly constant ionic equilibrium is maintained. The extent of permeability is different even among stenohaline Crustacea; low permeability has been noticed in animals that show euryhaline tendencies, while true euryhaline animals are even much less permeable. *Carcinus*, for example, is less permeable to ions in both directions than marine crabs like *Portunus* and *Hyas* (Nagel, 1934); however, it is permeable to both water and salts as shown by the dilution curves of Margaria (1931), Bateman's (1933) conclusion that the gill membrane is almost impermeable to water being incorrect (Krogh, 1939; Webb, 1940). In the freshwater *Cambarus bartoni*, Maluf (1937) has shown that the gills are permeable to water but not to electrolytes, but in a later paper[1] (1939*b*) he admits that chlorides could pass through in both directions. The gills of *Eriocheir sinensis* are permeable to water and to substances like chloride and ammonia (Krogh, 1939). There is possibly no aquatic crustacean the gills of which are not permeable to water, for even the brine shrimp *Artemia* is permeable as indicated by experiments with heavy water (Krogh, 1939). The experiments made on the permeability of the gills of *Leander* and *Palaemonetes*, though only of a qualitative nature, show that the gills are much less permeable than in marine or even euryhaline animals like *Carcinus*. Changes in osmotic pressure and water content during the moult cycle also support this conclusion. The condition observed in these prawns is comparable to that of *Eriocheir* and possibly *Cambarus*; definite permeability in both directions exists for water and ions. However, the amount of water that enters the prawns when they are in osmotically inferior surroundings must be much less than that observed in freshwater Crustacea with salt-reabsorbing mechanism, since large-scale urine production will not be consistent with conservation of salts. Loss of water from the tissues when the prawns are in hypertonic media is inevitable, though the amount of water lost is not considerable owing to low permeability. If the loss of water is great, as for example in very concentrated media, it is probably made up by taking in sea water through the alimentary canal; but there remains the question of excretion of salts, the site of which has not been definitely proved, though circumstantial evidence seems to point to the gills.

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[1] The value may be well below this in warmer latitudes (vide Panikkar, 1940*b*) as shown by Vialli's (1925) results.

The alternative possibility is an active transport of water (but not salts) into the body from the surrounding sea water. The osmotic work required to absorb water against an osmotic gradient would be of about the same order as that required for secreting salts to osmotically superior surroundings. It has to be mentioned, however, that active transport of water alone through a living membrane and against an osmotic gradient has not been demonstrated in any marine animal. Studying the ionic regulation of *Carcinus*, Webb (1940) brings circumstantial evidence in favour of water transport. That absorption of water takes place in Crustacea after moult is well known from the results of Baumberger & Olmsted (1928), Robertson (1937) and Drach (1939). In *Leander*, changes in water content and osmotic pressure during the moult cycle indicate the selective absorption of water immediately after moult so as to account for a rise of almost 5 % (cast included) in water content and a fall in osmotic pressure of about 0·2 % NaCl. If water and salts were absorbed without preference we should find a rise in the concentration of the blood after moult and not the gradual reduction actually observed, assuming, of course, that the excretory function remains normal. Since the highest osmotic pressure registered (3·030 % NaCl) is well below the value of sea water, this absorption takes place against an osmotic gradient and is, I think, sufficient proof of the ability of water transport in *Leander*, at least soon after moult. It is quite probable, therefore, that this capacity is not completely lost even during intermoult periods, and that active transport of water, probably across the gill membrane, plays a significant part in osmoregulation. Though technical difficulties in demonstrating this have not been completely overcome, it is hoped that analysis of ionic regulation will throw some light on this problem, for osmoregulation is mainly the result of the intensification of the processes at work in ionic regulation (Pantin, 1931; Webb, 1940).

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[1] Based on abstract in *Biological Abstracts*. The original paper was not available for consultation owing to the war.

The concept of salt transport, presumed by Schlieper (1929, 1935), conclusively shown in *Carcinus* by Nagel (1934) and explained on the basis of active ion absorption demonstrated by Krogh and his collaborators, accounts for the ability of many euryhaline and freshwater animals to maintain a high osmotic concentration. Intake of salts by *Palaemonetes* and *Leander* has been demonstrated. Though the possibility of at least some of the salts being absorbed through the gut cannot be ruled out, the quantity thus absorbed must necessarily be small owing to the fact that it would be unfavourable to the animal from the point of view of water economy. The only other place where salt assimilation can take place is the gills, and in view of the results obtained by Krogh (1939) and Koch (1934) the gills are presumably the site of ion transport in prawns.

Role of the Gills in Osmoregulation

From what has been said above, it will be clear that the gills of palaemonid prawns are the most vital organs in osmoregulation, especially in view of the relatively unimportant role played by the renal organs. They should have a low permeability to reduce the amount of osmotic work to be done when prawns are in hypotonic as well as in hypertonic media; they have to discharge salts to the exterior when the medium is hypertonic or alternatively transport water into the body from the surroundings, and they have to perform the function of ion assimilation when the medium is hypotonic. Confirmation on these points is possible only after the direct histochemical observations on gills, which are being made, have been completed.

The following facts in regard to the structure of the gills of Palaemoninae are relevant in this connexion. Allen (1892*a*), in his account of the minute structure of the gills of *Palaemonetes varians*, describes the occurrence of numerous reticulous and clear glands on the axis of the gill. Cuénot (1895), who observed similar glands in certain other Crustacea, considered them to have the function of mucus secretion; but Yonge (1932), from a comparative study of similar structures, concluded that these tegumental glands are responsible for the secretion of the cuticle of arthropods. He finds the cuticle thickest where the glands are most numerous. Considering the fact that it is the cuticle that reduces the permeability of chitin, the presence of a large number of such glands on the gills is suggestive of the role they play in securing the low permeability of these prawns. In the freshwater *Palaemon*, Patwardhan (1937) mentions that the axis is protected by a thick layer of cuticle and that the gill plate itself is covered by a double layer of cuticle. From an examination of the gills of *Carcinus*, *Maia*, *Hyas* and *Eriocheir*, Webb (1940) concludes, however, that the cuticle is of little significance in controlling permeability and that the control is really exercised by the nature of the epithelium. That the gills have an excretory function is suggested by the fact that dyes injected into the animal are collected by certain cells lining the blood vessels of the gills[1] (Kowalevsky, 1889), and Cuénot (1895) has observed that their behaviour is almost identical to that of cells of the end-sac. According to Allen these cells surround the veins and possess vacuoles with excretory concrements. Whether or not they have a chloride-secreting function cannot be asserted at present, but they are definite evidence of active excretory processes taking place in the gills. In regard to ion absorption, Koch (1934) has demonstrated the curious affinity for silver salts of certain cells found in the branchiostegites of *Leander serratus* and *Palaemonetes varians*, and he suggests from a comparative study of similar cells in other arthropods that this property is evidence of active ion absorption taking place in the branchial epithelium.

Role of the Kidneys in Osmoregulation

The mechanisms employed to achieve osmotic independence of the surrounding medium are quantitatively and qualitatively different in the different groups of animals which have succeeded in establishing themselves in fresh water. Those which have developed the capacity to produce hypotonic urine have in general the lowest concentration of blood, in contrast to those producing blood isotonic urine which, though found in fresh water, have a high osmotic concentration of blood. Maluf (1938), reviewing the question of excretion in the Arthropoda, concludes that the nitrogenous wastes, which in Crustacea consist mostly of ammonia (Delaunay, 1931), are probably secreted into the lumen of the antennal kidneys by the cells of the labyrinth. That the actual filtration of water and crystalloids from the blood takes place first of all into the coelomic sac is evident from the work of Picken (1936) and Peters (1935). The morphological differences between the antennal glands of the marine *Homarus* and the freshwater *Potamobius* emphasized by Peters, and the significant changes in chloride content which he observed in the different parts of the green gland, have established conclusively that the nephridial canal of the freshwater crayfish is mainly concerned with the reabsorption of salts from the urine as it flows down from the labyrinth into the bladder. It has been found that the nephridial organs are usually provided with longer nephridial canals in the freshwater as compared with marine Crustacea, as shown by Schwabe (1933) in the freshwater *Gammarus pulex* and the marine *Gammarus locusta*. Exceptions to this rule have been found in crabs, such as *Eriocheir sinensis* and *Potamon*[1] *fluviatilis*, which though capable of living in fresh water have excretory organs similar to those of marine crabs (Schlieper, 1929; Schlieper & Herrmann, 1930). To these must now be added the palaemonid prawns which, though capable of penetrating into fresh water, do not have an excretory system specially modified for the purpose. The blood-isotonic urine in *Leander serratus* and *Palaemonetes varians* is not surprising when we consider the structure of the excretory organs, for neither has a urinary canal. Both the end-sac and the labyrinth are small and compact and very near the external orifice of the bladder. The structure of the bladder epithelium precludes the possibility of any active reabsorption taking place there. However, there is a distinctive feature in the excretory system—the enormous renal or nephroperitoneal sac which absorbs dyes injected into the animal and necessarily performs a certain amount of active excretion (Weldon, 1891; Marchal, 1892). From the fact that the walls of the sac are richly provided with blood vessels it would appear that a certain amount of filtration may possibly also take place there. Though the essential lay-out of the excretory systems in *Leander* and *Palaemonetes* is similar to that of the marine prawns *Pandalus*, *Hippolyte* and the shrimp *Crangon* (Weldon), they are enormously developed and fused to form a large storehouse of urinary fluid. This would seem to be advantageous in the production of large amounts of urine, since the rate of urine production is likely to be different when the prawns are in different media. Schwabe (1933), who examined sections of *Palaemonetes varians microgenitor* (brackish water) and *macrogenitor* (fresh water), could not discover any noticeable structural difference in the excretory organs of the two varieties. Similarly the description of the excretory organs of the freshwater *Palaemon* given by Patwardhan (1937) is essentially the same as that of *Leander*. On anatomical and osmotic grounds the evidence available is that adaptation to fresh water in the Palaemoninae has not been accompanied by the development of a salt reabsorbing part of the nephridium and the ability to produce urine hypotonic to the blood. It is probably this very fact which has enabled the freshwater palaemonids to tolerate salt water.

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[1] I have repeated these experiments and obtained similar results.

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[1] Formerly known as *Telphusa*.

Evolutionary Significance of the Osmotic Behaviour of the Palaemoninae

The osmotic behaviour of *Palaemonetes varians*, *Leander serratus* and *L. squilla* appears to be of great significance when viewed in relation to the habits and distribution of the group to which they belong. The family Palaemonidae includes four subfamilies, of which the Pontoniinae are marine, the Desmocaridinae and Typhlocaridinae are fluviatile, while the Palaemoninae include the following seven genera with habitats ranging from fresh to salt water:

Pseudopalaemon	Two species. Marine.
Brachycarpus	Two species. Marine.
Leander	About fifty species. Mostly marine; some estuarine; a few fresh water.
Palaemonetes	About fifteen species. Mostly estuarine or brackish water; a few fresh water.
Palaemon[1]	About seventy-five species. Almost entirely fresh water; a few estuarine.
Euryrhynchus	Two species. Fresh water.
Cryphiops	One species. Fresh water.

The genera of the subfamily have been discussed in some detail by Kemp (1925). Leaving out of consideration the exclusively marine and freshwater genera, we find that species of *Leander*, *Palaemonetes* and *Palaemon* occur both in salt and fresh water. *Leander* is mainly marine, but a good many species are estuarine; at least two occur only in fresh or brackish water (*L. gardineri* and *L. fluminicola*); not less than five species are known only from fresh water (*L. potitinga*, *L. cubensis*, *L. modestus*, *L. capensis* and *L. annandalei*); others, like *L. concinnus* and *L. paucidens*, seem to be indifferent to the salinity of the medium. Kemp observed *L. fluminicola* nearly 700 miles up the Ganges in India. Of the four species of *Leander* found on the English coasts, *L. longirostris* is known to ascend many miles up rivers (Gurney, 1923; Schnakenbeck, 1933). *L. serratus* is able to survive in brackish water for short periods and *L. squilla* is often observed in brackish water, though both are essentially marine species confined to the littoral zone. *L. adspersus* is a marine and brackish-water species.

Palaemonetes, though found only in fresh and brackish water under natural conditions, includes at least two species that can thrive in sea water. *P. vulgaris* is mainly an estuarine species but is also known from the sea on the American coast (Faxon, 1879). As mentioned before, there are few, if any, valid records of *P. varians* from the sea, but the prawn has been observed to live for many months and to breed in the sea-water tanks of the Plymouth Laboratory.

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[1] Inclusive of the species referred by some carcinologists to the genera *Macrobrachium* and *Bithynis*.

Palaemon is an essentially freshwater genus, but a few species, such as *P. rudis*, *P. carcinus*, *P. malcolmsoni* and *P. lamarrei*, are known to migrate into brackish water during breeding periods (Kemp, 1915; Panikkar, 1937); the last-mentioned species is particularly tolerant to salt water and has been noticed in true brackish-water habitats (Panikkar & Aiyar, 1937). Menon (1938) has found the larvae of *P. rudis* and *P. carcinus* in brackish water.

It should be mentioned in this connexion that there are few reliable characters to distinguish *Palaemon* from *Leander*. While the main difference between the two is the presence of the hepatic spine in *Palaemon* and of the branchiostegal in *Leander*, there are two species (*L. potamiscus* and *L. fluminicola*) which, though obviously *Leander*, are without the branchiostegal spine, while in *Palaemon hildebrandti* the hepatic spine is sometimes absent; *P. mirabilis*, though technically a *Palaemon*, is remarkably like some species of *Leander* (Kemp, 1925). The specific characters of some of these species of *Palaemon* and *Leander* so overlap that their diagnosis is extremely difficult even for the specialist. Kemp, reviewing the species, considers that the genus *Palaemon* is probably polyphyletic in origin. The same view is held in regard to *Palaemonetes* (Kemp, 1925, p. 315), whose resemblance to certain groups of species of *Leander* in the adult as well as in the developmental stages is so marked that Gurney (1939) suggests there is nothing in the development of *Palaemonetes* to justify its separation from the genus *Leander*.

The discovery of osmotic independence in a purely marine species of *Leander* and of a highly developed homoiosmotic behaviour in *L. squilla* and *Palaemonetes varians* suggests that all the prawns known from varied habitats, included in the genera *Palaemon*, *Leander* and *Palaemonetes*, possess a well-developed mechanism of osmoregulation. Osmotic independence must naturally exist in all the freshwater genera of the Palaemoninae—*Palaemon*, *Euryrhynchus* and *Cryphiops*. The fact that all species of *Palaemonetes* are found either in fresh water or in brackish water, considered in the light of the behaviour of *P. varians*, would indicate that relative osmotic independence is probably to be found in all the members of this genus. It is to be expected that a similar independence will be found in all the freshwater and at least all true brackish-water species of *Leander*. In regard to marine *Leander*, direct evidence has been obtained only in *L. serratus* and *L. squilla*, but I consider that osmotic independence is likely to occur in *L. longirostris*, as judged by its migratory movements, and in many Indian species, such as *L. styliferus*, which I have often found in brackish and sea water. The degree of hypotonicity when in sea water is bound to be varied; it may even be completely absent when the prawns are in normal sea water and may become apparent only in concentrated sea water as observed in *Eriocheir sinensis* (Conklin & Krogh, 1938). It would be of the utmost interest to know the osmotic behaviour of palaemonid prawns from different habitats in different parts of the world, for we might then secure data on their physiological adaptation which would throw light on their evolutionary history.

If an animal shows a high degree of osmotic independence relative to the external medium, especially if it involves ability to maintain hypotonicity, it is reasonable to consider that the animal must at some time in its evolutionary history have inhabited fresh water (Pantin, 1931; Baldwin, 1937), for such a mechanism would never have been needed for a purely marine life. The penetration of most marine animals into brackish water is closely associated with the development of osmoregulatory powers; but we find in them only an ability to maintain hypertonicity when the external medium is dilute sea water. This is brought about by decreased permeability to water and salts and active transport of ions from the outside to the inside. Thus, animals like *Carcinus maenas* can survive in brackish water of very low salinity, and maintain a high osmotic concentration of blood (Schlieper, 1930, and others), but isotonicity is established the moment they are taken back to sea water (Frédéricq, 1904; Duval, 1925). A further development of this ability to maintain hypertonicity enables a brackish-water animal to penetrate into fresh water. The adjustments required would either be increased ability to assimilate ions and a low permeability to water, or the development of renal salt-reabsorbing mechanism whereby loss of salts would be reduced to a minimum. We have found that in *Palaemonetes* acclimatization to fresh water has been brought about by the former method without any undue specialization of the excretory system, and it is by virtue of this that the prawn is able to live in external media of a wide range of concentration, a feature which it shares with many other Palaemoninae.

Two theories are possible to account for the osmotic independence shown by *Leander serratus* and *L. squilla*. One is that they have been derived from prawns that have been established in fresh water and developed a high degree of osmotic regulation, but that for some reason they began to penetrate back into brackish water and then into the sea. In the same way that a low permeability was advantageous in reducing the entry of water into the body of prawns when in fresh water, it was helpful in reducing the loss of water when the medium became hypertonic. The second possibility is that the present-day species of *Palaemonetes*, *Palaemon* and *Leander* are descendants of prawns which left the sea for life in inlets that eventually became land-locked and were thus subject to increase in salinity; in consequence they were obliged to develop a mechanism for maintaining hypotonicity and a low permeability. An argument against the latter theory is that these genera are world-wide in their distribution, and it is unlikely that these special conditions could have been so widespread. Both possibilities may have occurred and may account for the suggested polyphyletic nature of the genera in question. I consider the first explanation the more plausible in regard to *L. serratus* and *L. squilla*, since, if it were otherwise, the optimum value of total osmotic pressure would certainly have been higher than 2·8 and 2·6 % NaCl. Whether all *Leander* have had a freshwater ancestry or not cannot be asserted until the numerous other marine species have been subjected to close study. Investigations on

Brachycarpus and *Pseudopalaemon* would be of exceptional value, for if they are stenohaline they may represent forms which have not developed osmoregulatory powers in their history; on the other hand, if they have an osmoregulatory behaviour comparable to that of *Leander*, they would represent forms that have become secondarily marine in recent times.

The biochemical aspect of adaptation to fresh water emphasized by Needham (1930, 1937) throws some light on the question of the recolonization of salt water suggested in *L. serratus* and *L. squilla*. One important condition to be fulfilled if an animal is to establish itself in fresh water is that it should provide, within its egg, enough ash for complete development instead of having to depend upon the environment for inorganic substances essential for the organization of the embryo. The provision of ash in the embryo is therefore as necessary to enable a species to establish itself in fresh water as osmoregulation is to enable the individual to live in fresh water. It is well known that there are many animals which though they occur in fresh water have not overcome this embryonic obstacle, and are thus obliged to migrate into salt water in which alone their eggs can develop. This migration may also be necessitated by the absence or inefficiency of the osmoregulatory mechanism in the embryo, the mechanism, however, attaining its full functional significance in the adults which can therefore migrate into fresh water. Gurney (1923) found that in *L. longirostris* the eggs can develop only in salt water in spite of the adults being quite at home in fresh water.

It is well known that a large egg and a reduced or suppressed larval development is a characteristic of many freshwater animals, but the information we possess on this point in the Palaemoninae is widely scattered and not as precise as could be desired. In the crayfishes, the Astacidae and Parastacidae, the eggs are always large and the young are liberated in a form closely resembling the parents. In *Palaemon*, some species, such as *P. lamarrei*, have large eggs and a reduced development (Henderson & Matthai, 1910), while in others the eggs are small and there is no doubt a long series of larval stages. Some species with small eggs, such as *P. carcinus*, *P. malcolmsoni* and *P. rudis*, are known to migrate from fresh water to brackish water in order to liberate their young, and from this circumstance it is to be concluded that the osmoregulatory mechanism is absent or inefficient in the early stages but becomes functional after growth has proceeded and the young prawns are ready to ascend the rivers. In all probability, however, it would be incorrect to say that all species of *Palaemon* with small eggs must have access to brackish water during the breeding season; for it is likely that small-egged species are to be found in localities so far inland that regular access to the coast is an impossibility. In such species it is to be assumed that an osmoregulatory mechanism is functional from the earliest stages.

In the freshwater prawns belonging to the Atyidae, a family much more primitive than the Palaemonidae and therefore, as one may suppose, established in fresh water for a still longer period, the eggs vary greatly in their size, and there are many small-egged forms which are certainly unable to migrate into brackish water.

What little we know of the reproductive habits and life history of *Palaemon* is sufficient to show that relative independence of the medium during developmental stages has not been attained at least in some species of an essentially freshwater genus, and it seems that although osmotic adaptation for freshwater life was achieved by the adults of *Leander*, a similar independence of the environment was not attained during larval life for embryonic or osmotic reasons. The habit of liberating the larvae in salt water, in an environment involving less osmotic work, may have brought about the slow suppression of the migratory habit, at least in some species of the Palaemoninae, until they became secondarily established in brackish water and finally in the sea.

SUMMARY

1. The brackish-water prawn *Palaemonetes varians* and the marine prawns *Leander serratus* and *L. squilla* are hypotonic in normal sea water, the blood of these species showing osmotic pressures equivalent to 2·3, 2·8 and 2·6 % NaCl respectively, in an external medium of 3·5 % NaCl.

2. *Palaemonetes varians* is isotonic in water of about 2·0 % NaCl and the species is practically homoiosmotic, the difference in its osmotic pressure over a range of 5·0 % NaCl in the external medium being only 0·8–1·0 %. The species has a very wide range of tolerance from water that is nearly fresh to concentrated sea water equivalent to 5·2 % NaCl.

3. *Leander serratus* is much less homoiosmotic than *Palaemonetes*, and has a limited tolerance to dilution and concentration of the environment. Homoiosmoticity is maintained up to a dilution of 2·5 % in the external medium when isotonicity is reached; but in lower dilutions there is a steady decline in osmotic pressure and the regulatory mechanism evidently breaks down.

4. The osmotic behaviour of *Leander squilla* is very similar to that of *L. serratus*, but the homoiosmotic behaviour is more marked and it has greater tolerance to dilution of the environment.

5. When *Leander* and *Palaemonetes* are transferred to very dilute sea water, the internal osmotic pressure falls gradually for about 14–24 hr., varying according to the size of the individual. After the lowest value has been registered there is a slight rise, and a steady state is thereafter maintained.

6. Studies on the changes of weight of prawns when transferred to diluted media indicate that the integument (gills) is permeable to water and that, at least in *Leander serratus*, the amount of water entering is mainly responsible for the dilution of the blood. There is a similar fall in weight when prawns are transferred to concentrated media, due to loss of water.

7. The urine of *Palaemonetes* and *Leander* is nearly isotonic with the blood irrespective of the nature of the external medium in which the animal is placed. This suggests that the kidneys do not play a significant part in the

osmoregulation, not even in examples of *Palaemonetes* acclimatized to nearly fresh water.

8. The contents of (1) the dorsal sac, (2) the nephroperitoneal sac, and (3) the urinary bladder of *Leander serratus* have been separately removed and the osmotic pressures determined. They do not present significant differences; hence a salt reabsorbing or a water reabsorbing mechanism is absent in the excretory organs.

9. The osmotic changes during the moult cycle of *L. serratus* have been studied. There is a slight rise in the osmotic pressure of prawns about to moult and the value comes down to normal a few days after the moult. A prawn of fresh weight of 1 g. absorbs about 0·3 g. of water during moulting.

10. The gills of palaemonid prawns are definitely permeable to water in both directions and, to a slight extent, to salts. As compared with stenohaline animals, however, the permeability of the gills of *Leander* and *Palaemonetes* is shown to be very low, and especially so in *Palaemonetes*.

11. Both *Palaemonetes* and *Leander* drink the external medium as judged by experiments with dyes. This behaviour is, however, erratic and occurs even when in hypotonic media. The evidence is insufficient to assume that water drinking is essential for osmoregulation.

12. The prawns are able to assimilate ions from dilute solutions presumably through the gills. Part of the absorption of salts may also take place through the gut wall.

13. The possible occurrence of some salt-excreting mechanism is emphasized; probably in the gills. It has not been cytologically proved, but attention is drawn to the excretory cells occurring in the gills. The osmoregulatory role of the gill in the light of its histological structure is discussed.

14. Palaemonid prawns seem to achieve osmotic stability by active absorption of ions when in hypotonic media and there is strong circumstantial evidence for active transport of water against the osmotic gradient, probably very effective when in hypertonic media. The osmotic work required for adjustment is brought to a minimum by the low permeability of the integument (gills), which gives the prawns considerable powers of salt retention.

15. The significance of the osmotic behaviour of *Palaemonetes* and *Leander* in the evolutionary history of the Palaemoninae is discussed. It is suggested that *Leander serratus* and *L. squilla* are probably species that have taken secondarily to marine life.

ACKNOWLEDGEMENTS

I have great pleasure in acknowledging my indebtedness to Prof. D.M.S. Watson for his continual interest and advice in this work and the hospitality of his department where the work was begun and the necessary apparatus assembled. My thanks are due to Prof. A.V. Hill for permission to work in his laboratory to learn the thermoelectric method and to Mr J.L. Parkinson for technical advice and assistance. To Dr E.J. Allen I am deeply indebted for his advice and criticism throughout the course of the investigation, and also for the loan of his sections of *Palaemonetes*. Dr W.R.G. Atkins has also been most helpful in discussing problems of osmosis. Finally, I am very grateful to Dr S.W. Kemp for the generous facilities given me for this work at Plymouth and his criticism of the paper; his intimate knowledge of problems of distribution has been of invaluable assistance. The work was carried out during the tenure of an Overseas Scholarship of the Royal Commission for the Exhibition of 1851.

Addendum

While this paper was in the press there has appeared an account of the osmotic pressure relations of nine species of crabs of the Pacific coast of N. America (Lowell L. Jones, 1941. *J. Cell. Comp. Physiol.*, Vol. XVIII, pp. 79–92). Of special interest is the fact that *Uca crenulata* shows remarkable powers of regulation and is even hypotonic in sea water, its behaviour being very much like that of the semi-terrestrial crabs examined by Pearse (*vide* Table I, p. 319). *Pachygrapsus crassipes* has been found to produce urine isotonic with blood. In *Hemigrapsus*, which has an osmotic pressure curve similar to that of *Carcinus*, a higher temperature seems to induce a slightly higher osmotic pressure. The author brings some evidence to indicate that crabs showing ability to maintain hypotonicity in sea water are better adapted for terrestrial life than others capable only of hyperosmotic regulation.

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THE ECOLOGY OF SOME PARASITIC COPEPODS OF GADOIDS AND OTHER FISHES

By N. G. Sproston and P. H. T. Hartley

From the Plymouth Laboratory

(Text-figs. 1–2)

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INTRODUCTION

During the study of the ecology of some fishes in the Tamar estuary in 1935–7 by one of us (Hartley, 1940), certain data concerning parasitic copepods of gadoids were collected, and as these were unpublished it was thought profitable to combine them with similar observations made during the period 1939–40 by the other author on gadoids caught in the sea nearby. The latter material was from two distinct habitats: (i) those fish caught in the trawl by the laboratory motor-boat *Gammarus* fishing within the limits of Plymouth Sound, together with those trawled by the *Salpa* in August 1939 from the Shagstone Grounds, which, although outside the Sound, are of similar depth (not more than 5 fathoms) and can be included with it under 'inshore waters'; (ii) other fish obtained from commercial trawlers fishing outside Tor Bay, Start Bay and over Rame Muds. The depth at which the latter were obtained is unknown, but probably all were from water between 10 and 30 fathoms in depth; these grounds are considered as 'offshore waters'.

An analysis of these combined data throws some light on the distribution, and the factors controlling it, of some of the commoner copepod parasites of gadoids.

LERNAEOCERA BRANCHIALIS (LINN.) (FAM. LERNAEOCERIDAE)

The Relation between the Migratory Habits of Gadus merlangus *and* G. pollachius *and their Infection with* Lernaeocera branchialis

Length-frequency tables relating to *Gadus merlangus*, *G. pollachius* and *G. luscus* caught in the estuaries of the Tamar and the Lynher during 1936 and 1937 have already been published (Hartley, 1940, pp. 47–51). Though the numbers of fish were rather small, the results tend to show that these gadoids enter the estuaries when they are a few months old and remain there for some 10 months, growing rapidly in the spring and early summer and less so in the autumn and winter. Throughout the estuarine period there seems to be a gradual leakage of the stock out to sea, but the main exodus usually takes place in the following spring.

Lernaeocera branchialis is a large blood-feeding parasite found projecting from the gill chamber of our more important food species of gadoids, *Gadus morrhua*, *G. merlangus* and *G. pollachius*, but only the last two are common at Plymouth. The irregular distribution of this parasite and its effect on the young stock seemed worthy of investigation. We had at our disposal records of superficial examinations of over a thousand specimens of the two commonest gadoids from the Tamar estuary which were caught in the tuck-net during 1936 and 1937, and more detailed dissections of 245 of the same species from the sea nearby caught in the trawl during 1939 and 1940.

The chief points brought out by these data are: (i) that the young fish migrating inshore are free from parasites and become infected after entering inshore or estuarine waters, and (ii) that infected fish tend to behave abnormally and do not return to the open sea with the main stock.

Gadus merlangus

This species was twice as common as *Gadus pollachius* and *G. luscus* in the Tamar estuary; *G. pollachius* was also infected with *Lernaeocera branchialis* in the Tamar, but length data of infected fish are only available for *Gadus merlangus*, and these are set out in Table I A, and in histogram form in Fig. 1. The correlation between the migratory habits of this species and its infection with *Lernaeocera branchialis* in the Tamar estuary is indicated by the following summary of the facts illustrated in Fig. 1.

Recruitment of the small fish of year-class 0, with length range 4–10 cm., took place in May, but none of these young fish was infected. Though over 300 gadoids of less than 10 cm. long have been examined, no fish less than 11·0 cm.

Table I. Infection of *Gadus merlangus* with *Lernaeocera branchialis* and *Clavella uncinata*

A. *From the Tamar Estuary,* 1936 *and* 1937 (*P.H.T.H.*)

Month	Total fish examined	No. over 10 cm.	A.M.L. for month	With *Lernaeocera*		With *Clavella*	
				No.	A.M.L.	No.	A.M.L.
1936							
i	41	41	20·14	9	20·16	1	(19·7)
ii	48	48	20·68	0	—	0	—
iii	16	16	19·13	8	20·63	0	—
iv	4	4	20·30	4	20·30	0	—
v	27	5	8·0	0	—	0	—
vi	5	0	(6·3)	0	—	0	—
vii	23	9	8·5	2	(19·0)	0	—
viii	68	64	12·05	9	12·94	20	12·35
ix	12	12	14·25	3	14·5	2	(14·5)
x	9	9	15·85	6	17·5	2	(13·5)
xi	7	7	16·5	5	17·0	1	(14·5)
xii	4	4	16·5	3	16·8	1	(15·5)
1937							
i	4	4	18·5	4	18·5	0	—
ii	1	1	18·5	1	(18·5)	0	—
iii	1	1	15·5	1	(15·5)	0	—
iv	0	0	—	0	—	0	—
v	0	0	—	0	—	0	—
vi	189	37	8·34	1	(11·5)	0	—
vii	65	58	12·0	3	14·5	0	—
viii	66	66	14·4	1	15·5	0	—
ix	49	49	15·8	5	16·1	1	(15·5)
x	10	10	18·6	1	(19·6)	1	(14·5)
xi	55	55	18·9	10	18·61	3	17·5
xii	11	11	16·2	1	(16·5)	1	(14·5)
Total	715	511		77		33	

B. *From Inshore and Offshore Waters near Plymouth,* 1939 *and* 1940 (*N.G.S.*)

Month	Origin	Total fish examined	No. over 10 cm.	A.M.L. for month	With *Lernaeocera*		With *Clavella*	
					No.	A.M.L.	No.	A.M.L.
1939								
viii	Sh.	24	24	29·5	6	30·0	5	29·8
x	R.	7	7	30·0	0	—	1	27·5
xi	R.	4	4	27·5	0	—	0	—
xi	P.	2	2	(15·0)	0	—	2	(15·0)
1940								
v	P.	4	4	15·0	3	14·7	2	15·6
vii	T.	31	31	23·8	2	24·0	2	22·9
vii	P.	4	2	8·6	0	—	1	(11·7)
viii	R.	14	14	24·0	0	—	4	24·0
ix	P.	1	1	(11·0)	1	(11·0)	1	(11·0)
x	R.	9	9	24·9	0	—	0	—
x	St.	4	4	24·2	0	—	0	—
xi	R.	4	4	24·8	0	—	1	(23·5)
Tota		108	106		12		19	

Note. A.M.L. = arithmetic mean length; P. = Plymouth Sound; R. = Rame Muds area; Sh. = Shagstone Grounds; St. = Start Bay; T. = Tor Bay.

was found infected (i.e. after 2–3 months of estuarine life). A length of 10 cm. has thus been taken as the arbitrary minimum, and only fish larger than this are included in the following analyses.

May to August was the period of rapid growth during which time the stock became infected. Infection did not necessarily take place at the same time for all fish, though information as to the state of maturity of the parasites relative to the length of the host is lacking for the Tamar material. In August 1936, 9 out of 64 of the rapidly growing new stock were infected, or 13·1 % (for the whole period of rapid growth the infection was 9 out of 75 or 12·0 %). For the same period in 1937 only 5 out of 161 fish were infected, or 3·1 %. A period of less rapid growth followed, normally from September to the following spring, and ended in the run to sea; but in September 1936 though netting continued as before, there was a marked fall in the numbers of whiting caught. This may indicate a precocious run to sea of fish mostly less than 15 cm. in length. From September to December 1937 the slowly growing stock showed a four-fold increase in infection—17 out of 125, or 13·6 %. The 1935 stock persisted into the spring of 1936, and a similar persistence of the 1937 stock is hinted at, in spite of the irregularity in the numbers of fish caught towards

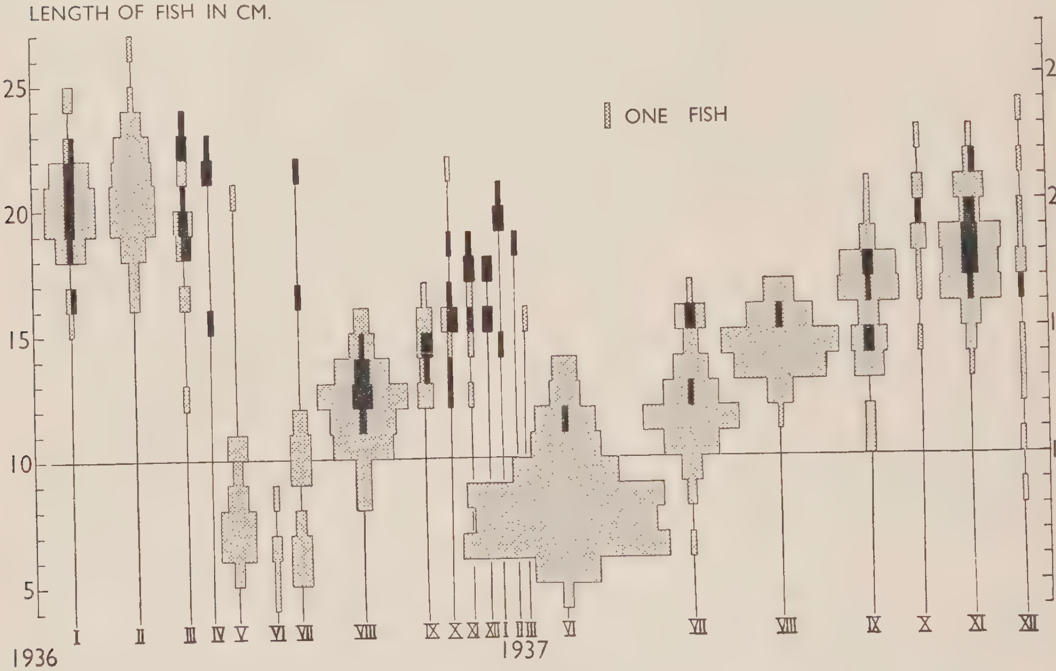

Fig. 1. Histogram representing the total fish examined from the Tamar estuary in 1936 and 1937. Infected fish shown black and uninfected fish stippled. Ordinates represent the length of the fish in cm. and the numerals along the abscissae indicate months.

the end of the year. Apart from the anomalous absence of infection in February 1936, the rate of infection over the first 3 months of 1936 was fairly high, 17 out of the 105 fish, or 16·2 %, which is of the same order as that obtained for the period of slow growth at the end of 1937.

Factors governing the run to sea are unknown, but it does not seem to be dependent on the attainment of a certain minimum size or on the availability of food (cf. the precocious run to sea of small fish in midsummer of 1936); however, at some time after the period of rapid growth there is a sudden fall in the numbers of fish caught: the 1935 stock fell 76 % in March 1936, and the 1936 stock fell 82 % in September of the same year. It was immediately after this exodus that the remaining fish showed a sudden increase in the rate of infection which had a tendency to approach 100 %. These large infected fish lingered in the estuary for some months after the arrival of the new stock.

A comparatively few *Gadus merlangus* were examined from inshore waters (Table 1 B and Fig. 2*b*), but about half of the small fish (year classes 0 and i) were infected, and they are evidently a counterpart of the lingering post-exodus fish of the Tamar which had failed to reach the sea with the main stock. In spite of the small number of fish examined from offshore waters, their freedom from infection with *Lernaeocera* is remarkable: in Fig. 2*c* the two infected specimens came from a haul 'off Tor Bay' and may possibly have been taken in the shallower waters of the bay itself. It may be concluded, therefore, that the year class i whiting leaving the rivers and making out to sea in the normal way are very rarely parasitized by *Lernaeocera*, nor do they become infected after reaching the deeper waters.

Gadus pollachius

This species was only a third as common as *G. merlangus* in the estuary but about three times as frequently met with as the latter species in Plymouth Sound (Table II). A similar length distribution throughout the year was found in the Tamar for pollack as for whiting, but the growing fish showed less regularity in numbers (Hartley, 1940, Table VIII). An outstanding difference is the later date—in June—at which recruitment took place, which is explained by breeding starting some weeks later than that of the whiting. The length distribution of inshore pollack (Fig. 2*a*) shows what at first sight appears to be a bimodal curve, but this may be due to the irregular breeding of this species, which in some years breeds so late that young only appear inshore in the winter. This must have been so in 1939 to account for the double banking of the histograms for 1940. It is a remarkable fact that, at Plymouth, small bronze coloured pollack were taken at all times, except during midsummer, in the Sound; these fish appeared to be in good condition, and the examination of their stomach contents showed that their diet consisted mainly of crustaceans, but that in the autumn and winter small teleosts had been eaten, also Mollusca and vegetable matter such as *Enteromorpha* and *Zostera*. In the spring, 10–15 cm. fish usually contained polychaete remains;

TABLE II. INFECTION OF *GADUS POLLACHIUS*

A. *From the Tamar Estuary,* 1936 *and* 1937 (*P.H.T.H.*)

Month	Total fish examined	No. over 10 cm.	No. with *Lernaeocera*
i	13	13	2
ii	19	19	0
iii	8	8	1
iv	1	1	0
v	0	0	—
vi	130	0	0
vii	48	48	0
viii	18	18	0
ix	4	4	0
x	10	10	3
xi	19	19	4
xii	17	17	1
Total	287	157	11

B. *From Inshore and Offshore Waters near Plymouth,* 1939 *and* 1940 (*N.G.S.*)

Month	Origin	Total fish examined	No. over 10 cm.	A.M.L. for month	With *Lernaeocera* No.	With *Lernaeocera* A.M.L.	With *Clavella* No.	With *Clavella* A.M.L.	With *Lepeophtheirus* No.	With *Lepeophtheirus* A.M.L.
1939										
viii	St.	1	1	(38·5)	0	—	0	—	0	—
ix	P.	1	0	—	—	—	—	—	0	—
x	R.	4	4	28·25	0	—	2	(30·0)	2	(28·0)
xi	P.	2	2	16·0	0	—	1	(17·5)	0	—
xii	P.	3	3	12·66	1	(12·0)	0	—	0	—
1940										
i	P.	3	3	11·58	0	—	1	(11·5)	0	—
i	P.	1	1	25·5	0	—	1	(25·5)	1	(25·5)
ii	P.	4	4	12·14	0	—	2	(12·4)	0	—
iii	P.	2	2	13·7	0	—	0	—	0	—
iv	P.	13	13	13·6	4	14·3	2	(14·3)	1	(14·0)
iv	P.	1	1	22·5	1	(22·5)	1	(22·5)	1	(22·5)
v	P.	10	10	14·5	3	14·5	3	14·7	0	—
v	P.	1	1	21·0	0	—	0	—	0	—
vi	P.	4	3	16·6	2	17·0	2	17·0	0	—
vii	P.	14	3	15·4	2	16·9	1	17·7	1	(17·7)
viii	P.	43	29	11·4	4	16·1	3	13·5	0	—
ix	P.	6	6	12·55	0	—	4	13·3	0	—
x	P.	3	3	13·0	2	12·75	1	13·3	0	—
xi	P.	20	20	13·8	1	(15·8)	8	14·4	0	—
xi	P.	1	1	21·0	1	(21·0)	1	(21·0)	1	(21·0)
Total		137	110		21		33		7	

A.M.L. = arithmetic mean length; P. = Plymouth Sound; R. = Rame Muds area; St. = Start Bay.

C. *Fish Infected with* Lepeophtheirus pollachii. *Inshore Waters,* 1939 *and* 1940 (*N.G.S.*)

Length range	No. fish examined	With *Lepeophtheirus* No.	With *Lepeophtheirus* A.M.L.	With *Lepeophtheirus* %
10–15 cm.	87	1	(14·0)	1·15
Over 15 cm.	25	4	19·2	16·0
Offshore fishes, 1939 *and* 1940 (*N.G.S.*)				
Over 15 cm.	4	2	(28·0)	—
Total fish over 15 cm.	29	6	—	20·7
All pollack	116	7	—	6·05

it was extremely rare to find a stomach empty. The lingering of pollack infected with *Lernaeocera branchialis* is better demonstrated from our records of inshore fish than from those from the estuary. Estuarine fish showed a rate of infection for the two years of only 7 % (Tables II A and III A): 10 of these 11 infected fish were taken after the period of rapid growth (September–March 1936–7), when the whiting from the same area showed a remarkably high degree of infection (59 %), the infection rate for pollack for the latter period alone being 16 %.

Only four large fish (year class ii) were brought in from over the Rame Muds, but none of these was infected (Fig. 2 *a*, 'offshore'). Pollack, therefore, like whiting, become infected during their inshore or estuarine phase, more particularly in the former habitat, according to our observations.

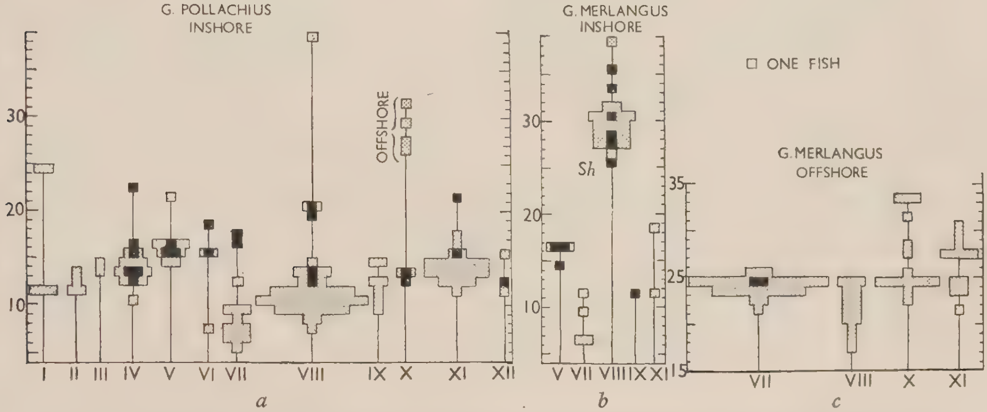

Fig. 2. Histograms representing the total numbers of fish examined from inshore and offshore waters near Plymouth during 1939 and 1940. Infected fish are shown black and uninfected fish stippled. Ordinates represent the length of the fish in centimetres (to the nearest half cm. above) and the numerals along the abscissae indicate months. *Sh*, fish caught from Shagstone Grounds, 1939.

The Relation between Parasitism and the Growth of the Fish

The causal trend of the correlation discussed above would seem to be that parasitism with *Lernaeocera branchialis* has some physiological effect on these gadoids which delays their seaward migration, rather than the fish remaining behind through some other set of circumstances and becoming parasitized owing to longer exposure to infection. The latter possibility can be tested by reference to Table III, in which the rates of infection for both species of fish are summarized according to their length groups for the three habitats. Owing to a significant number of *Gadus merlangus* being caught in the Tamar (A), the figures for this habitat are separated into the period of rapid growth (May–August) and the rest of the year when growth is slower (September–April). During the latter period the degree of infection is very nearly the same for both size groups, notwithstanding the small number of young fish, whereas of the 226 larger fish only 53 were infected. If infection were a simple function of the size of the fish, i.e. depending mainly on the volume of potentially infective water passing over the gills, and on the length of time the fish remained exposed to infection, then the larger fish would be expected to show a much higher percentage infection than the smaller, whereas this was not so. That the larger fish show three times the infection of the smaller in the first period is, however, explained by referring to Fig. 1 which shows that they are represented by the lingering of the old stock which is very heavily infected; this same fact is reflected in the annual rates shown in the last column of Table III A.

In *G. merlangus* from inshore waters the 31 % total infection is very high, but it is made up as already suggested by small fish lingering behind the main stock on their way to sea from the rivers. The group of larger fish (probably in their third year), shown above *Sh* in Fig. 2*b*, were caught on Shagstone Grounds in the squid trawl in no more than 5 fathoms of water, so they may be included in the inshore habitat: incidentally, they show about the same rate of infection as the larger fish from estuarine waters.

G. pollachius from inshore waters show five times the infection in older fish than in younger fish, when figures for the whole period are taken (Table III B): no seasonal separation is justifiable owing to the irregularity in length distribution and the small samples available. That this marked difference is due to the 'lingering' phenomenon is evident from Fig. 2*a*.

Discussion of the possible Physiological Factors influencing Infected Fish

The nature of the physiological changes in these two species of gadoids effected by the presence of *Lernaeocera branchialis*, and having their main outcome in the postponement of the run to sea, is unknown. There are several lines of approach to the problem of the direct effect of this parasite on its host, and some of these may be considered here. The *condition* of parasitized fish as compared with non-parasitized has been examined during the 1939–40 investigations; the condition factor $K=\frac{w\times 100}{l^3}$ (where w is the weight of the fish in grams and l is the length in centimetres) has been used. These figures for each fish are not reproduced here, for it was found that they showed no correlation whatever with infection. The value of K is considered unreliable when individual differences are sought among fish at very different growth stages. The greatest error, perhaps, is introduced by the gross weight of the fish which includes the highly variable stomach contents. Though there are no records published of any measurement of the condition of fish infected with this parasite, Andrew Scott (1929, pp. 106–8) states that during the heavy infection year of 1928 the young whiting from the shrimping grounds between Mersey and Dee showed so marked an emaciation that infected ones could be picked out at a glance. Thomas Scott (1909, p. 90) makes the statement that 'whitings and other gadoids have been captured reduced almost to skin and bone, having one or more large worm-like *Lernaea* [=*Lernaeocera*] hanging at their gills full of the red blood they had extracted therefrom'. Such emaciation has never been observed by us.

TABLE III. INFECTION OF *GADUS MERLANGUS* AND *G. POLLACHIUS* WITH *LERNAEOCERA BRANCHIALIS* SUMMARIZED ACCORDING TO THE GROWTH OF THE FISH

A. *From the Tamar Estuary,* 1937 *and* 1937 (*P.H.T.H.*)

Gadus merlangus

Length range	May to August			September to April			All months		
	No. fish examined	Infected fish		No. fish examined	Infected fish		No. fish examined	Infected fish	
		No.	%		No.	%		No.	%
10–15 cm.	216	11	5·1	37	8	21·6	253	19	7·5
15–25 cm.	32	5	15·6	226	53	23·2	258	58	22·3
Total catches	248	16	6·45	263	61	23·5	511	77	15·0

Gadus pollachius

No length data of infected fish from Tamar available.

No infection recorded during the period May–August; October–April: of the 82 fish examined above 10 cm., 11 were infected (13·4 %).

Infection over the two-year period = 7 %.

B. *From Inshore Waters,* 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Length range	No. fish examined	No. infected fish with *Lernaeocera*
10–20 cm.	8	4
Over 20 cm.	24	6
Total	32	10 (=31·2 %)

Gadus pollachius

Length range	No. fish examined	With *Lernaeocera*	
		No.	%
10–15 cm.	84	9	10·7
Over 15 cm.	22	12	54·5
Total	106	21	19·8

C. *From Offshore Waters,* 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus	*Gadus pollachius*
Two infected fish from 73 examined (2·7 %)	No parasitic copepods were found in the four fish (A.M.L. 28·25 cm.) examined in October

Had there been a retarding effect on the growth of the fish by the presence of the parasite it would have been evident in our records. In Tables I and II the arithmetic mean lengths of the monthly catch are given for comparison with the A.M.L. of the infected fish, and the unexpected correlation is found that, on the whole, the infected fish are larger than the uninfected, even when fish of the same size group are considered together, as they are for inshore and offshore fish. It is difficult to account for this anomaly, though two possibilities suggest themselves: first, that mere size is a favourable factor, a larger fish presenting a greater infective surface. Though this possibility was rejected above as an explanation of the higher rate of infection of large fish as a whole, it may possibly be operative here when a group of fish of a restricted length range, whether large or small, is being considered. The second possibility is that the smaller infected fish of each length group have been eliminated from the samples. Owing to the loss of blood (see below) and consequent reduction in respiratory efficiency, the weaker fish (i.e. those under the A.M.L. of the length group) may perhaps enter the surface water where the oxygen tension is higher; if so, they would not be caught in the trawl or in the tuck-net which operate over the bottom. Indeed they may, in these higher levels, become an easy prey for gulls and other predators.

Since infection has no measurable effect on these two species of gadoids at Plymouth, it would appear that they have, to a large extent, become tolerant of the parasite.

We have calculated that the capacity of the intestine of a fully grown *Lernaeocera* is at least 100 c. mm., which is considerable in relation to the blood volume of a young gadoid. It is not known at what rate the parasite feeds, i.e. how often this volume is extracted from the host, though it is shown elsewhere that feeding is not a continuous process (Sproston & Hartley, 1941). Granting, then, that the blood loss to *Lernaeocera* is probably far greater than that to any other parasite (that is, relatively and absolutely great), it would be of great interest to know the blood-cell counts of infected and uninfected fish; for in this way it might be possible to measure the effects of parasitism. Some degree of 'secondary anaemia'[1] is to be expected, for the loss of blood to the parasite would be made good by absorption of tissue fluids, via the capillary walls in the first instance, and finally equilibrated by absorption of water from the external medium (see below); the net result being a dilution of the tissue elements of the blood, and, perhaps, a greater or less strain on the haemapoietic organs. The various well-known symptoms of anaemia would contribute to a chronic cachexia which may well account for the parasitized fish failing to respond to the normal urge to run to sea.

A second possibility is tentatively suggested to account for the lingering of parasitized gadoids in estuarine and inshore waters: this concerns the osmoregulatory mechanism of the fish. The absorption of fluid from the external medium to make good the loss to the parasite is complicated by the fact that the gadoid is living in hypertonic media, whether it is in the sea or in estuarine waters. The salinities at various points in the estuaries of the Tamar and Lynher were determined by Milne (1938) who worked concurrently with one of us (P.H.T.H.) during the work on the Saltash tuck-net fishery there. It is notoriously difficult to determine the exact salinity level at which fish are living in an estuary, but in all probability these gadoids normally remain near the bottom where changes in salinity are not so great as elsewhere. Though these fish were taken in the upper reaches of both estuaries in summer, in winter they were only caught in the neighbourhood of Saltash Bridge where the salinity did not fall below 25–24 $^{0}/_{00}$; this represents the minimum bottom salinity recorded, and probably a similarly low figure would be obtained from certain places in Plymouth Sound (Barn Pool, for instance, where a large proportion of our gadoids were obtained during the latter series of observations). The large 'lingering' infected gadoids found in the seaward end of the estuaries in autumn and winter when the rivers are in flood are therefore inhabiting media of a much lower salinity than the normal fish of the same size which have migrated to offshore habitats.

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[1] 'Secondary anaemia' is used here in the clinical sense, to indicate the result of simple blood loss, in contrast to 'primary anaemia' which is defective blood-cell production.

It is well known that the blood of marine teleosts has an osmotic concentration of only about a third of that of sea water. From the work of Smith (1930) and Keys (1933) it is now clear that the mechanism of regulation consists of drinking sea water and excreting the excess of salt through the gills. Excretion of salt against an osmotic gradient necessarily involves the expenditure of considerable energy. To quote from Smith's review of the subject (1932, p. 11): 'a large fraction of the ingested and absorbed sea water is excreted extra-renally...nearly all the water excreted as urine must be procured from the sea water at the expense of considerable osmotic work.... Consequently the normal fish tends to keep the urine flow and the ingestion of sea water down to a minimum for the sake of physiological economy.' The choice of habitat may therefore be a physiological response to the necessity of absorbing additional sea water to maintain the normal blood volume (i.e. pressure equilibrium), and it is possible that to reduce the concomitant osmotic work the parasitized fish would tend to remain in media having the minimum salinity compatible with life.

It was hoped that these two hypotheses concerning the exact nature of the physiological upset of parasitized fish could have been tested experimentally; but so far it has been found to be impossible to keep the fish infected with *Lernaeocera* alive in the laboratory. This, in itself, is a pertinent fact. When batches of gadoids were brought into the aquarium only a few of the uninfected fish died in transit, but nearly always the infected fish would be found dead, floating venter upwards, with the operculum widely distended and the mouth open to the fullest extent; any surviving infected fish always died within an hour or so of being placed in the tanks. This, in fact, is the only direct effect of physiological upset observed by us in infected fish.

The Susceptibility of the different Gadoids to Infection with Lernaeocera *spp.*

From Table III it would appear that *Gadus merlangus* was twice as susceptible to infection with this parasite as *G. pollachius* in the estuaries, and that the susceptibility was 3:2 in inshore waters. When, however, our total figures for all habitats are taken together there is little difference between them:

Species	Total no. of fish examined	Total no. of fish infected	% infection
G. merlangus	616	89	14·5
G. pollachius	267	32	12·0
Both species	883	121	13·7

G. minutus migrates inshore during its first year, though from the data (Hartley, 1940, p. 50) it does not appear to ascend the estuaries with any regularity. Of the 27 specimens examined from the Tamar and the Lynher during 1936 and 1937, none was infected with copepods. During 1939 and 1940, 120 specimens were examined from the sea and were free from copepods with the exception of two fish, both well above the average size of our samples; one of them had a mature specimen of *Lernaeocera branchialis* attached to the anterior angle of the fourth gill arch. This is the first record of an adult of this species being found on *Gadus minutus*. Hesse (1870, and 1891, p. 191, as quoted by Wilson, 1917, p. 40) records what is considered to be a larval form on the gills of the same host. The occurrence of the parasite on this host seems to be rare, for *G. minutus* is quite as common as *G. pollachius* in inshore waters, and Ford (1931) considers it to be the commonest gadoid on the local trawling grounds outside Plymouth Sound; yet the previous investigators of parasitic copepods at Plymouth who must have examined large numbers of this fish have never recorded the presence of *Lernaeocera branchialis* on it.

Gadus luscus is infrequent inshore, but of the eleven specimens from Rame Muds, three were infected with *Lernaeocera lusci*. During the previous investigations (1936, 1937) 289 fish of this species were examined from the Tamar, of which 246 were over 10 cm. in length; but only two of these were infected with *Lernaeocera*, which, in all probability was *L. lusci*, though they were not identified at the time. This parasite is specifically distinct from *L. branchialis* (a parasite rarely found on *Gadus luscus*): apart from certain morphological characters it differs from the latter species in its position of attachment to the host, being found attached to the gill arches at about the middle of their length and penetrating only into the branchial vessels. The host reaction is also different to this species: at the 'neck' of the parasite the branchial mucosa of the host is hypertrophied: a traumatic overgrowth in the form of a swollen collar which obscures much of the body of the parasite. This has also been observed by Vanden Berghe (1933), but is not mentioned by any other observer. It is common to find multiple infections of this species; one of the fish from Rame Muds had six *Lernaeocera lusci*. Since so low an infection rate (0·81 %) was found for *Gadus luscus* in the Tamar, and a comparatively high one (25 %) for the offshore fish it would seem that for this species infection takes place after the run to sea. It is possible, therefore, that the intermediate host carrying the copepodid stages and the males is different from that of *Lernaeocera branchialis* (*Pleuronectes flesus*), which is an active vector mainly in estuarine and inshore waters; so that the whole life cycle of

L. lusci would seem to be passed in deeper offshore waters, a marked contrast to that of *L. branchialis* (see below). Scott & Scott (1913, p. 145) record *L. lusci* from *Gadus luscus* caught 10 miles off Aberdeen in January 1901.

Gadus morrhua is infrequent in the Tamar estuary, and only two out of the 20 fish examined from this habitat were infected with *Lernaeocera branchialis*; no fish of this species have been examined by us from the sea at Plymouth. A. Scott (1929, p. 108) says that the cod caught with the highly infected whiting were rarely infected off the Lancashire coast.

No *Lernaeocera* spp. were found by us on other gadoids: a few each of *Gadus virens* and *Urophycis blennoides* were examined from offshore, also 23 *Onos mustela* and a few each of *O. tricirratus* and *O. maculata* from inshore waters. Numbers of *Callionymus lyra* were examined from the estuary in 1936 and 1937, and some from inshore in 1939 and 1940, but they were free from copepod parasites.

The Duration and Distribution of the Developmental Phases of Lernaeocera branchialis

The life history of this parasite is well known, though the records of the developmental forms are not numerous in the literature. There is a free-swimming copepodid stage which becomes attached to the gill filaments of the flounder; here a series of modified copepodid stages are passed, finally giving rise to pelagic forms (redescribed by Sproston, 1941). The female is still cyclopoid in appearance but has an elongating genital segment; the male is similar to the infective copepodid. These pelagic forms remain within the gill cavity of the flounder for a short time, attachment being effected by the chelate second antennae; copulation takes place here and the male soon dies. The fertilized female leads a pelagic existence until it finds itself within the gill chamber of a gadoid; it then becomes attached near the anterior angle of the fourth gill arch and undergoes a peculiar metamorphosis. The long genital segment first twists and then dilates, and the cuticle becomes thicker. Meanwhile anchoring processes grow out from the sides and top of the cephalothorax, these becoming the bifurcating 'antlers' of the adult. Penetration continues, at first through the branchial vessels and then into the ventral aorta and often into the auricles. Occasionally fibrous and fatty tumours develop, but usually the only histological response is a fibrotic thickening of the vascular system around the anchoring structures. The suctorial form of mouth is situated on the end of a conical proboscis embedded in the blood vessel. The swollen genital segment becomes a darker red as the blood-feeding habit continues. Two very long thin egg strings are extruded just above the small cylindrical abdomen; they are tightly coiled in a boss-like tangle and can often be seen projecting with the abdomen and part of the genital segment from the operculum of an infected gadoid. Owing to the permanent mode of attachment, the 'antlers' remain in situ after the death and fragmentation of the rest of the parasite.

During the investigations by the first author (1939–40) data were collected with a view to determining the longevity of *Lernaeocera* and the age of the host when infection occurs. The development of the adult female on the gadoid has been separated, somewhat arbitrarily, into seven substages as shown in Table IV. The 'Pennella' stage was so called by Scott (1901) to denote that stage of the fertilized female before the antlers grow out and just prior to torsion.

TABLE IV. SUBSTAGES OF *LERNAEOCERA BRANCHIALIS* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

From Inshore and Offshore Waters near Plymouth, 1939 and 1940 (N. G. S.)

A. *Distribution of Substages Throughout the Year*

Month	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	Totals	
P	—	—	—	1	—	—	—	—	—	—	—	—	1 P	
U	—	—	—	—	1	—	—	—	—	—	—	—	1 U	19 (U+V+W)
V	—	—	—	—	1	—	1	—	1	—	—	—	3 V	
W	—	—	—	2	2	1	2	5	—	1	1	1	15 W	
X	—	—	—	2	2	1	1	7	—	1	—	—	14 X	16 (X+Y)
Y	—	—	—	—	—	—	—	1	—	—	1	—	2 Y	
Z	—	—	—	—	4	—	1	1	—	—	1	—	7 Z	

Symbols used to denote the substages found

P = 'Pennella' stage.

U = Very young form: torsion of body just complete, but genital segment not yet swollen. Pale red in colour.

V = Very young form: antlers showing first bifurcation and penetrating into the branchial vessels. Medium red in colour.

W = Young form: antlers penetrating as far as ventral aorta, but no external egg strings. Deeper red, especially in the abdomen.

X = Full grown form: external egg strings emerging but not ripe.

Y = Fully mature form: egg strings hatching or spent, and body very dark red in colour; abdomen almost black.

Z = Remains of dead antlers embedded in sublingual and vascular tissue.

B. *Distribution of Substages on Fish of different Size Groups*

Length range	No. of infected fish	No. of parasites	Sub-stages							Intensity of infection per fish		
										Single	Double	Triple
10–15	11	11	P		2V	5W	2X		Z	11	0	0
16–22	14	21		U		7W	6X	Y	6Z	9	3	2
Over 22	8	11			V	3W	6X	Y		5	3	0

Notes. (1) The minimum length *G. merlangus* infected was 11 cm.; *G. pollachius* 12 cm.

(2) Minimum length fish bearing dead parasite: *G. merlangus* 13·5 cm. and *G. pollachius* 15·5 cm.

(3) Of the 8 multiple infections found, 7 were composed of parasites in two distinct substages. Proportion of multiple infections: 8/33 (24·3 %).

(4) In all respects the parasites from the two host species were similar in their distribution, and for this reason the data are considered together.

The occurrence of these various substages throughout the year is shown (Table IV A), and the last column shows the relative abundance of these stages over the period of observation. It is seen that the immature and mature stages are scattered in time, so that no definite infection or breeding period can be assigned to *Lernaeocera*.

Stekhoven (1936) and Stekhoven & Punt (1937) have studied similar material from the cod from continental inshore waters at Helder, and they conclude that there is a simple annual cycle, that maximal infection of the intermediate host, the flounder, takes place in April and May, and that the mass infection of the cod is in midsummer. In their Table III (1937, p. 653) they give a summary of the details concerning *L. branchialis* from some dozen cod, all about 1½ years old. These data, however, do not seem to lend very convincing support to their conclusions, for the parasites were by no means of a similar age: about half of the number examined belonged to various immature stages (P to V in our nomenclature), and of the rest, about a half were mature and the remainder consisted of dead remains. Apparently the collections were made only in the months of May and September, but the results show what is probably a typical cross-section of any population of *Lernaeocera*: for, in general, they agree with our findings on the distribution of the substages throughout the year. Our conclusions are, however, very different from these authors', for the data we have show conclusively that generations are being produced continuously all the year round.

Table V shows that the few flounders examined between December 1939 and December 1940 were all infected with the copepod developmental stages except those two fish below 10 cm.

TABLE V. THE INFECTION OF *PLEURONECTES FLESUS* WITH THE DEVELOPMENTAL STAGES AND MALES OF *LERNAEOCERA*

(December 1939 to December 1940, N. G. S.)

Month	ii	ii	ii	iii	iv	iv	ix	ix	xi	xii	xii
Length of fish	12·2	13·4	13·5	9·0	10·8	11·5	12·1	17·3	9·8	22·5	12·3
No. of *Lernaeocera*	3	8	7	0	16	7	1	2	0	20	10

On other fish: *Cyclopterus lumpus*, caught inshore on 3. iv. 40, had three mature males of a lernaeoceran on the gills, and *Solea solea* (also a fully grown fish), caught off Roscoff (France) on 3. x. 38, had a single half-grown copepodid on the gills, which also belonged to this family of parasites (see Sproston, 1941).

All the flounders were caught in strictly inshore waters at Plymouth, and most of them from the seaward side of Laira Bridge in the Cattewater: Mr Spooner confirms our view that salinity and other conditions here are comparable with those near Saltash Bridge in the Tamar estuary. Records of hosts for the copepodid stages are sparse in the literature and they usually refer to the occurrence 'on flat fish such as the flounder' and similar vague terms. Claus and Metzger's original material came from the flounder as did Stekhoven's; it is curious that there are no records by the Plymouth workers at any time for these distinctive young stages. During the survey of the flounders in connexion with the Saltash Tuck-Net Fishery (P.H.T.H.) no special search was made, unfortunately, for gill copepods; indeed, there are no records extant for them from flounders taken in rivers or offshore, and the only localities mentioned are the inshore waters of Europe. It is also curious that so far no larval lernaeoceran has been recorded from the Western Hemisphere, though a species described by Wilson (1917), closely allied to ours, is common on gadoids of the Atlantic seaboard. We think that it is safe to state that the normal intermediate host at Plymouth is *Pleuronectes flesus*, for though a thorough microscopical examination has been made of the gills of 100 other flat fish caught at Plymouth between August 1939 and December 1940, no larval forms were found. The numbers of flat fish were distributed between nine species as follows: *P. platessa* 55, *P. cynoglossus* 1, *P. microcephalus* 2, *P. limanda* 21, *Rhombus laevis* 4, *Arnoglossus laterna* 3, *Solea solea* 9, *S. variegata* 1, and *S. lutea* 4.

It is regrettable that no flounders were obtainable during the summer months at Plymouth; but that breeding of the parasite was taking place during this time is evidenced by the occurrence of mature adults shedding nauplii (see Table IV A, substage x)—so that our evidence is complete for the continuous breeding of *Lernaeocera*.

By a careful examination of the facts at our disposal we can form some estimate of the length of the life cycle of this parasite. Eggs have been hatched in the laboratory in plunger jars containing sea water, and the pelagic copepodids were swimming actively on the second day. Some of these had attached themselves to the spent part of the egg strings, for want of other substratum, and had moulted, once at least, into the pupoid form which is described elsewhere (Sproston, 1941); this took place overnight and they lived for only a few hours afterwards. It is possible that these copepodid stages on the flounder are run through rapidly, for whenever seven or more were found together at least one was in the process of moulting. Stekhoven & Punt (1937, p. 653) consider that as the pelagic fertilized female is seldom encountered, it must usually pass directly from the flounder to the cod when the latter migrates inshore. This, in general terms, is borne out by our observations on other gadoids, but the term 'directly' is misleading. It is improbable that so small and ill-equipped a crustacean could survive planktonic conditions in the open sea, and finally sink to the bottom and seek out a suitable gadoid; it is much more likely that the cycle is completed in the shallow inshore waters and in estuaries; but any direct transmission by actual contact is most unlikely. That these pelagic fertilized females can exist as free animals for short periods has been shown by Andrew Scott (1901, p. 44), who collected them from filters placed over the outflow from the Peel flounder hatcheries: he says that he also caught males in this way, but their presence was accidental, since they normally die soon after copulation on the gills of the flounder. The ratio of males to females was 1:25. Up to the beginning of the century these forms had only been taken by I.C. Thompson (1893, p. 212, pl. 26, fig. 7) in tow-nettings off Puffin Island off the coast of Anglesey; the succeeding stages—the 'Pennella' stages of Scott—have since been found on the gills of gadoids, and recently Stekhoven & Punt (1937) have found an unbroken series (described in their Tables I and II). This series lacks, however, the extremely elongated form, showing the incipient antlers as small bulges on the cephalothorax, which was discovered by Thomas Scott in 1900 on the gills of a whiting in the Bay of Nigg, Aberdeen, and has never been found again (A. Scott, 1901, pl. IV, fig. 6). Regular tow-nettings have been taken outside Plymouth Sound throughout the year for a number of years, and Dr Lebour and Mr F. S. Russell, who have examined the plankton, inform us that they have never found a larval stage of *Lernaeocera*; this bears out our suggestion that a gadoid once out at sea does not become infected with *L. branchialis*.

For arriving at an approximation to the minimum length of the gadoid phase of the life cycle, the following rationale was employed. Since the youngest gadoid found infected was 11·0 cm. long and the youngest bearing a dead parasite was 13·5 cm., then the whole phase may be completed while the fish is growing some 2·5 cm. From the histograms this time may be estimated, very roughly, at 8 weeks.

Additional data in support of this are obtained from the age of epizootic organisms. In August 1939 a third-year whiting was caught on the Shagstone Grounds bearing two *Lernaeocera*: one was immature (stage V) and the other, with half-spent egg strings, was covered with an epizootic hydroid. We are indebted to Mr W. J. Rees for examining this at the time and for his report on the specimen, which is as follows:

> On the exposed gravid genital segment (the so-called body) of the *L. branchialis* there was a well developed colony of *Clytia hemisphaericum* (Gronovius) = *Clytia johnstoni* (Alder, 1857). The hydrocauli were branched and numerous gonothecae were present containing medusa buds. The colony was normal in every respect and there was nothing to suggest that it was parasitic on the copepod. It was probably at least 6 weeks old and may have been two or three months old.
>
> Many examples of a triple association between a fish, a parasitic copepod and a hydroid are known. Jungersen (1911) cites several instances and also describes a hydroid, *Ichthycocodium sarcotretes* occurring on *Sarcotretes scopeli* Jungersen, 1911 (Lernaeoceridae). This hydroid he regards as semi-parasitic because the polyps possess no tentacles and no nematocysts.

A. Scott (1929, p. 108) gives the following information regarding epizootics: 'The *Lernaeocera* apparently live quite a long time. I have had specimens overgrown with zoophytes and young mussels 1·3 mm. long.' The length of mussel spat at the time of settlement appears from the records to be rather variable as it depends on local conditions; Matthews (1913, pp. 557–9) indicates that laboratory-bred spat settled when lengths were between 1 and 2 mm., but that one young larva collected from the plankton had not settled though it was 4 mm. long. Dr Lebour has kindly given us her opinion on the probable length of time during which Scott's specimens had been attached; this, she considers, may have taken place shortly before they were collected, and in any event they could not have settled more than a month previously.

Returning to Table IV, the relative frequency of the occurrence of the adult substages throughout the year may be taken as an approximate measure of the relative duration of those substages. The suggestion, in general terms, is that the frequency with which any phase is encountered in a regular and continuous sequence is proportional to the time occupied by that phase in the sequence. For instance, of the 36 living *Lernaeocera* found on the two species of gadoids, 20 were immature and 16 were mature, so that approximately five-ninths of the time is spent in the immature condition and four-ninths of the time in the mature condition. Similarly, one-seventh of their time is taken up in penetrating from the outer to the inner vascular tissues, growing the antlers and completing the torsion of the body (substages P to V), the rest of their time being divided about equally between continuing the growth of the genital segment, both in length and girth, and the final stage during which the egg strings are extruded and the ripe eggs shed. After this the animal remains alive for a short time (only two Y were found, so that it is fairly rare, i.e. probably a brief stage); death is followed by rupture and fragmentation of the projecting parts, leaving the antlers and some part of the 'neck' embedded in the tissues of the host.

Two direct observations on the living *Lernaeocera* are noted here as they have some bearing on the time factors of these substages. There was usually no difficulty in keeping the adult parasites alive in circulating sea water in the laboratory provided they were in no way injured during the dissection from the host. One W stage had been under observation for some hours when it started to extrude egg strings; after about 3 hr. approximately half the normal bulk of egg strings were extruded, but the eggs were pale and there was no sign of nauplii in the distal ones. At this time, unfortunately, these observations had to be concluded owing to an air-raid. Other egg-bearing females have been kept under observation for some days; in one the nauplii were hatching from the distal eggs as these became detached, over a period of 4 days' observation, and though at the end of this time the animal was sacrificed for another experiment, there was no marked diminution in the mass of the remaining egg strings. Though no measurements have been made to determine the rate of shedding of the eggs it seems that this is protracted over, perhaps, 2 or 3 weeks. Under natural conditions the eggs may be shed more rapidly owing to the projection of the end of the genital segment of the ripe female into the surrounding water, a little beyond the operculum of the fish.

Owing to this protracted shedding of ripe eggs the metanauplii from the first eggs liberated may well have passed through several, or even all, their stages on the flounder by the time the last sister egg has just hatched out. Nevertheless, it is unlikely that the same flounder is the recipient of the whole gamut of larvae from one female *L. branchialis*, though superficially the facts suggest this: for in all the infected flounders examined bearing seven or more larvae, some of the latter were males and females in the fertilization stage and some were newly arrived copepodids which had not yet become attached by the rostral filament, but were merely hanging on to the gills by their chelate second antennae. This variety of stages probably represents infection from several parents; this is an acceptable view considering the extremely high infection rate of the flounders from the seaward end of estuaries.

From the above account it seems probable that *L. branchialis*, at least at Plymouth, has about four or five consecutive generations a year, and that these generations show an infinite degree of overlap.

Multiple Infections of Gadus merlangus *and* G. pollachius *with* L. branchialis

The records of multiple infections of fish caught in the sea near Plymouth are shown in Table IV B: and since seven out of eight of those recorded within the limits of inshore waters consisted of parasites of distinctly different ages, any possibility of immunity is precluded. This has already been demonstrated by the similar findings of Stekhoven & Punt (1937, Table III). That the chances of reinfection increase with the age of the fish (provided that the latter remains inshore or in the estuary) is demonstrated by the absence of reinfection in fish less than 15 cm. long, and its presence to the extent of 24·3 % in fish over 15 cm. long from inshore waters. In Table VI similar results from the estuary are given as far as our records permit. The multiple infections are shown here also for both host species together for comparison with those from the sea. One fact which is difficult to explain is that in whiting from the Tamar there is no increase of multiple infection with age of the fish as shown above. The other outstanding result is the remarkable agreement in each fish and in each habitat of the proportion of multiple infections to the

TABLE VI. DISTRIBUTION OF *LERNAEOCERA BRANCHIALIS* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

Intensity of Infection per Fish from the Tamar Estuary, 1936 and 1937 (P.H.T.H.)

Length range	Infected fish	Multiple infections		No. of parasites per fish			
		No.	%	1	2	3	4
G. merlangus:							
Under 15 cm.	19	5	26	14	5	0	0
Over 15 cm.	58	14	24	44	7	6	1
Total	77	19	24·7	58	12	6	1
G. pollachius:							
Total fish	11	3	27·3	8	1	2	0
Both species	88	22	25	66	13	8	1

total number of infections (about a quarter). Considering the number of fish examined, this figure is perhaps rather high; and it is regrettable that larger numbers of fish were not examined from both habitats so that the results could have been treated statistically, to test the possibility of infection being a predisposing factor for reinfection.

CLAVELLA UNCINATA (O. F. MÜLLER) (FAM. LERNAEOPODIDAE)

Some Factors Influencing its Distribution on Gadus merlangus *and* G. pollachius

The distribution of *Clavella* shows, in some respects, a marked contrast to that of *Lernaeocera*, particularly in relation to the inshore and offshore migrations of the hosts. Its occurrence month by month in the three habitats on *Gadus merlangus* and *G. pollachius* is shown in Tables I and II respectively. The incidence on fish of known length has been examined for *G. merlangus* in the estuary (P.H.T.H.) and for both species from the sea (N.G.S.), but as there is no very important correlation these details are not reproduced here. The seasonal occurrence is at first sight significant in the estuary, for it was found on *G. merlangus* only during the last third of the year; this, however, is more likely to be a length correlation, since a similar seasonal variation was not found inshore.

TABLE VII. INFECTION OF *GADUS MERLANGUS* AND *G. POLLACHIUS* WITH *CLAVELLA UNCINATA* SUMMARIZED ACCORDING TO THE GROWTH OF THE FISH

A. *From the Tamar Estuary*, 1936 *and* 1937 (*P.H.T.H.*)

Gadus merlangus

Length range	No. fish examined	With *Clavella* No.	With *Clavella* %
9·5–15 cm.	253	25	9·9
Over 15 cm.	258	8	3·1
Total	511	33	6·45

Gadus pollachius

Of the 157 fish examined only one was infected with *Clavella* (in November 1936): 0·64 %

B. *From Inshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Length range	No. fish examined	With *Clavella* No.	With *Clavella* %
9·5–15 cm.	4	3	(75)
Over 15 cm.	29	8	27·5
Total	33	11	33·3

Gadus pollachius

Length range	No. fish examined	With *Clavella* No.	With *Clavella* %
9·5–15 cm.	87	20	23
Over 15 cm.	25	11	44
Total	112	31	27·7

C. *From Offshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Of the 47 fish examined, all of which were over 20 cm., eight were infected: 17 %

Gadus pollachius

Of the four fish examined two were infected (A.M.L. = 30·0 cm.)

When the results are summarized according to the growth of the fish (Table VII), some interesting factors suggest themselves: first, the marked difference in the infection rates of estuarine and inshore fishes. The total infection rate of *Lernaeocera branchialis* on the two species of host in the estuary and inshore was 13·2 and 22·5 % respectively, whereas for *Clavella uncinata* it was 5 and 31 %. That the increase in infection with *Lernaeocera branchialis* inshore is due to the 'lingering' effect of parasitized fish being intensified, has already been suggested. Parasitism with *Clavella*, however, does not seem to have any such repercussions on the migratory habits of the host, and the six-fold increase in infection of inshore fish as compared with those from the estuaries seems to be brought about by a different set of factors. The most probable is its lower tolerance to dilution of the external medium. Experiments described elsewhere (Panikkar & Sproston, 1941) have shown that *Lernaeocera branchialis*, by virtue of a normal osmotic concentration below that of sea water, can tolerate dilution of the external medium at least down to 2·24 % NaCl, which is probably lower than that normally tolerated by the host. Unfortunately, no experiments on the osmotic behaviour of *Clavella* have been made, but casual observations have repeatedly shown that it dies quickly when very little tap water is added to the sea water in which it will live for some hours in the laboratory. *Lernaeocera*, on the other hand, has been kept alive under laboratory conditions (isolated from the host) for over a week on several occasions.

An important paper published recently (1939) by Poulsen, on the biology of *Clavella uncinata*, contains a great deal of interesting information; the distribution of this parasite on the cod in Danish waters was extensively investigated and it was found to be fairly common, especially in the Cattegat and in the Belt Sea, but infection decreased with salinity and Poulsen considers that of 16 ‰ to be lethal. He also remarks on the difficulty of determining the exact salinity level at which fish are found; from his maps (Figs. 6, 7) it seems that the relatively highly infected fish of the Belt Sea area were caught in 10–20 m. of water, or even beyond the 20 m. line. Even if fish were confined to the bottom layers where salinity is highest, this is appreciably lower than the mean bottom salinity in the Tamar estuary. Poulsen quotes Jacobson's figures for the Belt Sea at 20 m. as 20–26 ‰, but the bottom salinity of the Tamar estuary where other species of gadoids were found does not fall below 24 ‰ even in winter. It may be that a local race of *C. uncinata* has become adapted to the more brackish conditions of the Danish waters. It would be interesting to know the distribution of *Lernaeocera* spp. in the same region.

Poulsen found that no year-class o fish were infected, but that the highest infection occurred in the year-class i fish (38 %), thence falling steadily as the age advanced. The smallest cod found with *Clavella* was 11·0 cm. long, and this Poulsen includes in year-class i. There is a possibility that the sudden increase in infection in the yearling cod may be correlated with their migratory habits: a relatively high minimum salinity being the limiting factor. Graham (1934) discusses the state of our knowledge of the migration of young codling and he considers that, while it is true that there is a winter visitation of these young fish into inshore waters, Meek's theory (1916) of a periodic (annual) in- and offshore migration is by no means proven. He thinks it more likely that there is a general scatter of the post-larval phase and, since they seem to like a rough bottom where crustacean food is abundant, any fish entering inshore waters may remain there provided that the bottom is suitable, in spite of other inclement factors; though Graham was careful to point out that the data in his possession were insufficient to make more than a working hypothesis. If the young codling are in inshore Danish waters, where the salinity is lower than elsewhere, then it is possible that the absence of *Clavella* on the year-class 0 fish may thus be accounted for, and that they become infected after their first offshore migration.

Among the infected *Gadus merlangus* in the Tamar estuary there was one small fish (8·8 cm.) which was certainly in its first year; the smallest inshore infected fish measured 9·6 cm. and as this was the only one infected below 10 cm., 9·5 cm. was taken as the arbitrary minimum, and only fish above this length are considered in the following analyses. From Table VII it is seen that in the estuary the infection of *G. merlangus* dropped to a third of its intensity after the period of maximum growth, but on the other hand, inshore *G. pollachius* showed nearly double the infection after the period of maximum growth. We can offer only one explanation for this result; perhaps the decreasing salinity conditions met with by the growing fish in the river as the season advanced were such as would be unfavourable to the developing nauplii, so that few succeeding generations were forthcoming to reinfect the stock. However this may be, it seems that conditions in the Sound were altogether more favourable to the parasite, for here the degree of infection is of a similar order to that found by Poulsen in Danish waters for the allied species of host of the same age.

The susceptibility of the various species of gadoids to infection with *Clavella* is similar to that with *Lernaeocera*, except that we have not found the former on *Gadus luscus*; one specimen of each parasite was taken from *G. minutus* from the sea but none from the river. In spite of the unequal distribution of *Clavella* on the gadoids of the estuary (Table VII A), the total infections from our combined records are not very dissimilar:

Species	Total no. of fish examined	Total no. of fish infected	% infection
G. merlangus	591	52	8·8
G. pollachius	273	34	12·5
Both species	864	86	10·0

The Duration and Distribution of the Later Developmental Phases of Clavella uncinata

Unlike *Lernaeocera*, *Clavella* has only a single host in its life cycle, so that the hazards of the free living stages are enormously reduced as compared with those of the former type of parasite. Perhaps as a direct consequence of this

Clavella produces fewer eggs. Gurney (1934) has given the first and only account of the complete developmental cycle of this copepod. He had considerable difficulty in rearing copepodids from eggs under laboratory conditions at Plymouth, but later he was able to collect a series of intermediate forms from gills of local pollack.

Some 150 gadoids have been examined throughout the year by one of us (N. G. S.), but the pupa-like developmental stages described by Gurney have not yet been found. The various adult substages have, however, been found in several months of the year. As Gurney did not suggest any grouping of these substages, we have tentatively divided them into three as indicated in Table VIII; in section A of this table their distribution is shown throughout the year.

TABLE VIII. SUBSTAGES OF ADULT *CLAVELLA UNCINATA* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

From Inshore and Offshore Waters near Plymouth, 1939 and 1940 (N. G. S.)

A. *Distribution of Substages Throughout the Year*

Month	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	Total
X	1	—	—	—	—	—	2	13	3	—	8	—	27 X
Y	—	3	—	1	1	—	2	8	2	1	11	—	29 Y
Z	2	—	—	2	4	2	2	18	3	13	13	1	60 Z

Symbols used to denote the substages of the 'adult' parasite

X = all very young forms in which the genital segment has not swollen and is about one-third of the body in size.

Y = young immature forms in which the genital segment has swollen but in which the egg strings have not emerged.

Z = mature forms with external egg strings, including those with spent egg strings.

B. *Distribution of Substages on Fish of Different Size Groups*

Length range	Infected fish	No. of parasites	Substages			Multiple infections		
						No.	%	No. fish (No. parasites)
9·5–15	22	29	10 X	11 Y	8 Z	4	18	18 (1), 3 (2), 1 (3)
16–20	11	26	6 X	7 Y	13 Z	4	36	7 (1), 1 (2), 1 (3), 1 (5), 1 (9)
Over 20	15	62	11 X	11 Y	40 Z	8	53	8 (1), 2 (2), 1 (3), 1 (7), 1 (9), 1 (10), 2 (11)

Notes. (1) Minimum length of *G. merlangus* infected 11·0 cm.; *G. pollachius* 9·6 cm.

(2) Minimum length of *G. merlangus* with Z stage 11·5 cm.; *G. pollachius* 14·0 cm. No dead and disintegrating parasites were found.

(3) Among the multiple infections in the three groups the following proportions of fish had dissimilar stages occurring together: smallest $\frac{1}{4}$, medium $\frac{3}{4}$ and largest $\frac{4}{8}$.

(4) As with *Lernaeocera* the two hosts showed similar distribution of parasites in all respects.

There is a slight indication of two main breeding seasons, though the number of specimens is insufficient to warrant a definite conclusion. The youngest adult substage occurs throughout the summer and autumn but is absent in the spring, but the maturing forms (Y) were found all the year round, though the mature forms (Z) were always more abundant, with a concentration in summer and autumn of about four times the average monthly distribution. It is not likely that the few Y group individuals recorded in the spring were remnants of the previous year's brood, for we have every reason (see below) to think that these substages are passed through fairly quickly; it is more probable that they represent an early brood of the same year: the X substages corresponding to these were not found by us. Using the same approximation as was employed above for *Lernaeocera* in order to form a rough estimate of the incidence in time of these substages, it will be seen that in the last column of Table VIII A the numbers of immature forms (X and Y) together are about equal to the number of mature (Z) forms, also that the numbers of X and Y are nearly equal. Thus, it is probable that the time spent in completing the X and Y phases is nearly the same, and that each is about half that of the mature phase Z. The smallest whiting and pollack infected in inshore waters, from which these data were mostly collected, were respectively 11 and 9·6 cm. in length, and the smallest infected with a mature egg-bearing *Clavella* were 11·5 and 14·0 cm. Accordingly, at least in the whiting, the X to Z substages are passed through within a few weeks, perhaps about a month at a rough approximation. From Table VIII A it thus seems that reproduction takes place throughout the year as it apparently does also in *Lernaeocera*.

In Table VIII B it is seen that the number of mature egg-bearing females increases with the length group of the fish, i.e. it is in accordance with the probability of infection increasing with the time of exposure of the host under favourable conditions for infection. (It was assumed that estuarine conditions were not so favourable, hence the decreasing infection rate with age of fish—vide Table VII A.) Further, the rapid increase in the numbers of Z forms may be due to the longer persistence of this stage, causing, in effect, a 'piling up' of this final stage on the older fish.

Multiple Infections

The increasing percentage of multiple infections with the length group of the fish from the sea (Table VIII B) clearly demonstrates that there is no immunity to superinfection developed by the fish. In half of the multiple infections the parasites were of markedly dissimilar ages (see note 3, Table VIII). In the third-year whiting caught offshore it was common to find a large number of parasites of graded ages, and in some of them there were no mature Z forms; so that these successive stages must represent superinfection from other fish. It is quite probable that autoinfection occurs on one host, for spent females have also been found in company with very young X forms.

The state of maturity of the parasites found on the gadoids from the Tamar

TABLE IX. DISTRIBUTION OF *CLAVELLA UNCINATA* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

Intensity of Infection per Fish from the Tamar Estuary, 1936 and 1937 (P.H.T.H.)

All of the 33 fish infected were less than 15 cm. long, the smallest infected fish was 8·8 cm.

No. of parasites per fish:	1	2	3	4	
No. of fish infected:	26	4	2	1	Multiple infections: 7/33 or 21·2 %.

estuary was not determined, but data were collected regarding multiple infection and are given in Table IX. Since all the infected fish from this habitat were of the first length group dealt with from the sea, they are comparable and, indeed, they do show a similar rate of superinfection (21 % as compared with 18 %).

Direct Effects on the Host resulting from Infection by Clavella

As with *Lernaeocera*, the values for the condition factor, *K*, of the hosts have been determined for fish infected with *Clavella*, but as they show no correlation whatever with infection, they are not reproduced here. Similarly, there is no consistent variation in the arithmetic mean lengths of monthly groups of fish infected and not infected with *Clavella* (see Tables I, II); so as far as our data go, it may be said that *Clavella* has no appreciable effect on the host.

Clavella is much smaller than *Lernaeocera* and it seldom occurs in very large numbers; moreover, it is probably mainly a mucus feeder, though it may also feed on detritus and on gill tissue. In the pollack its habitat is distinctive, and in all specimens except one the parasites were found on the tips of the gill filaments, and usually on the first gill of each side (though sometimes also on the second gill), and situated towards one or the other end of the gill rather than in the middle region of the gill arch. When full-grown parasites were found on the pollack the adjacent four or five gill filaments, both on the gill to which it was attached and on the next gill, were shorter and appeared to have been browsed down to the extent of one-fifth or one-sixth of the length of the normal filaments. These browsed gill filaments did not, however, show a marked anaemia, though they were usually adherent, suggesting a hypersecretion of mucus resulting from the irritation and feeding of the parasites. Dawes (1940) has made a much-needed contribution to our knowledge regarding the histological effect of trematode gill parasites on their hosts, and though no such detailed investigations have been made in the present study, it seems quite possible that the effects are comparable. Poulsen (1939, p. 241, Fig. viii) gives a figure of an eroded fin of a cod caused by the browsing of *Clavella*: this shows a whitish edge which is thickened on the eroded margin. He considers that the effects on the gills would cause a more vital injury, and that in both instances the mechanical presence of the parasite would affect the movements or reduce the efficiency of the respiratory current in the branchial cavity. Hence, he suggests that *Clavella* may have a distinct detrimental influence on the growing stock, since at least in Danish waters the incidence of infection is fairly high. He has also determined the condition factors for infected and uninfected fish, but his results do not show any significant correlation. His explanation is that those fish taken to be uninfected have probably been infected in the recent past, and their lowered condition factor would produce a spuriously low mean value among the 'uninfected' fish.

The *Clavella* found on the whiting were never attached to the gill filaments:

on the smaller fish they were invariably found attached to the gill rakers on the first gill and more rarely on those of the second: they are actually more common on the small spinose bosses on the inner margin of the gill arch, though they are also found quite frequently on the long rakers on the outer margin of the first gill. In the older whiting, however, it was not unusual to find *Clavella* also attached to the roof and sides of the buccal cavity and sometimes on the tongue, but they were rarely found on the inner face of the operculum and never (as they are on the cod) on the outer surface of the body. Though there is no specific difference between the *Clavella* infecting the two common gadoids at Plymouth (Gurney, 1934) there is a distinct influence exerted in some way by each kind of host which determines the characteristic habitat taken up by the infective copepodid of the *Clavella*.

It may be added that the whiting may be expected to suffer much less from the presence of this parasite than the other gadoids, for the situation of the parasite is usually well removed from soft tissues and, on this host, it must be largely a detritus feeder.

THE GENUS *LEPEOPHTHEIRUS* (FAM. CALIGIDAE)

Like all other caligids, the species of *Lepeophtheirus* have a single host in their life cycle; in this they resemble *Clavella*, but unlike the latter they are not permanently attached to the host. As a group they are among some of the least modified of parasitic copepods. They are primarily marine, and owing to their thin cuticle, which may be subservient alike to excretion and gaseous exchange, they are unable to withstand marked or rapid changes in salinity of the external medium. Their sensitivity to reduced salinity when the host enters more or less brackish waters is illustrated by the distribution of *L. pollachii* and *L. pectoralis* at Plymouth.

Gurney (1933, p. 334) quotes Hutton's observations on *L. salmonis*. Hutton has found the parasite 85 miles from the sea and has shown that it can withstand fluviatile conditions for two weeks or more; but this is exceptional, and the fact that the egg strings very soon break away from the females denotes that conditions are certainly not suitable for breeding. The females are said to be less viable than the males. Gurney endorses the popular belief that their presence on a fish is strong evidence that it is freshly run from the sea. The present records do not include this species.

L. pollachii may have been overlooked in the samples taken from the Tamar estuary as no special search was made for it; but it was occasionally taken during 1939 and 1940 from the seaward end of the estuaries and from Plymouth Sound, as well as from offshore habitats, so that it is capable of withstanding a slight dilution of the external medium. Details of its occurrence are given in Table II C.

Perhaps the chief factor in limiting its distribution to the larger fish is its rigorous preference for a peculiar habitat: it is invariably found attached to the under-side of the tongue of pollack, though sometimes it occurs in other positions in the sublingual space after the death of the host. It has never been found on the operculum or outer surface of the body, and never on any other gadoid. Chalimus stages have not been found. Its absence from the smaller fish is therefore explained by the habitat being too small to accommodate it. There is no obvious seasonal variation in numbers, but our collection was too small to study this species from the point of view of breeding phases.

L. pectoralis is usually considered a very abundant parasite on pleuronectids, and unlike the foregoing species shows no marked preference for a particular species of host. Its habitat is not quite so constant as that species, though it is usually found in the neighbourhood of either of the pectoral fins. That it is even more sensitive to dilution of the external medium is shown by its absence at all times from *Pleuronectes flesus* and *P. platessa* caught at the seaward end of the estuaries at Plymouth, although it was found occasionally on these fish caught in the Sound. Over 2500 flounders and a considerable number of plaice from the Tamar estuary were examined by one of us (P.H.T.H.) during 1936 and 1937, but not a single specimen of *Lepeophtheirus pectoralis* was found, though a careful search was made for them. A single (20 cm. long) specimen of *Pleuronectes flesus* caught in the Sound harboured this species. The incidence of infection of *P. platessa* in the Sound, as estimated from casual observations, seems to be lower than that usually met with in offshore waters. The low percentage of infection in our records is probably due to the majority of our fish being caught at the seaward end of the estuaries; these fish were young (0–i year class).

TABLE X. INCIDENCE OF *LEPEOPHTHEIRUS PECTORALIS* ON *PLEURONECTES PLATESSA*: 'INSHORE'

Year class	0	i	ii	iii	iv	Total
Length range (cm.)	Up to 9	9–18	18–24	24–30	Over 30	
No. of fish examined	2	26	11	6	9	54
Length of infected fish	—	12·5	—	24·0	34·0, 38·0	4 fish
Month of capture	—	Jan.	—	Aug.	Oct. and Feb.	7·4 %

NOTES ON THE DISTRIBUTION OF *LERNAEENICUS* SPP. ON THE SPRAT

This genus of parasites belongs to the same family as *Lernaeocera* and may have similar powers of toleration to brackish water. No sprats have been examined from the sea during the present investigations; but the following records, made during the study of the Saltash tuck-net fishery of the Tamar estuary (Hartley, 1940, p. 42), are included here for comparison with the related *Lernaeocera* on gadoids from the same habitat. As was found with *Lernaeocera*, the several species of *Lernaeenicus* have characteristic habitats which are adhered to rigorously: *Lernaeenicus sprattae* is always found on the eye and *L. encrasicola* is embedded into the body musculature, the head sometimes reaching through to the coelom. The distribution of infected specimens of *Clupea sprattus* with the two species of *Lernaeenicus* is summarized in Table XI. The incidence of infection is rather low, and about a third of the infected sprats probably belonged to the stock of the previous year. This may be another instance of the lingering inshore of fish infected with parasites of this family. It is also interesting to note that the 8·0 cm. fish caught in September 1936 had a triple infection of *Lernaeenicus spratttae* on one eye. In this host, therefore, as in gadoids, there is no immunity developed.

TABLE XI. INFECTION OF *CLUPEA SPRATTUS* WITH *LERNAEENICUS* IN THE TAMAR ESTUARY, 1936 and 1937 (P. H. T. H.)

Year	No. of fish examined	With *L. sprattae* No.	With *L. sprattae* %	With *L. encrasicola* No.	With *L. encrasicola* %
1936	477	5	1·12	1	0·21
1937	899	4	0·445	2	0·22
Total	1376	9	0·655	3	0·22

Lengths of fish infected with Lernaeenicus

Date	A.M.L. for sample	Lengths of infected fish: With *L. sprattae*	Lengths of infected fish: With *L. encrasicola*
1936			
23 May	8·78	10·1	8·4
19 Aug.	4·88	5·4	—
18 Sept.	4·82	6·8; 8·9; 8·0	—
2 Oct.	7·25	7·5	—
1937			
21 Jan.	5·21	—	6·6
1 Feb.	4·92	5·1	—
April	5·33	5·8	5·5
8 May	6·02	5·7	—
27 July	3·63	3·6	—

Neither *L. sprattae* nor *L. encrasicola* is very common round our coasts.[1] Scott & Scott (1913, pp. 156–9) mention a catch of 600 sprats from the shrimping grounds off Blackpool which yielded a 2·3 % infection with *L. sprattae*. Leigh-Sharpe (1935, pp. 270–5, Figs. 1–7) redescribes and figures these two parasites from material obtained from the Tamar estuary in the spring of 1934; he obtained 29 from 960 fish (2·9 %), and he states that Dr Gurney examined a catch of over a thousand fish from the same locality at about the same time and obtained a similar percentage of infection. He does not record any multiple infections, though among Scott's material one fish had three on one eye.

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[1] Mr Ford recalls examining large catches of sprats at Plymouth among which some specimens had black holes in the eye, which he took to indicate the sites of previous infection with this parasite. Unfortunately these specimens were destroyed by enemy action, but the possibility remains that the real rate of infection of sprats is higher than the apparent rate given above.

L. encrasicola is even less common. A. Scott (1907, p. 94) examined some hundreds of sprats caught off Blackpool in February 1906 and only found a single infected fish, and Leigh-Sharpe found only one among the 960 fish from the Tamar; the 1936 and 1937 catches from the same locality yielded a slightly higher percentage (0·2 %). We have no data as to the condition of the infected sprats, but observers state that *L. sprattae* causes blindness, which would indirectly lead to malnutrition and possible death; both species would cause a certain amount of debility through loss of tissue fluids on which they feed. These combined effects may explain the rarity of these two parasites.

Summary and conclusions

The migratory habits of *Gadus merlangus* and *G. pollachius* are examined in relation to the variations in the rate of infection of these fish with *Lernaeocera branchialis*. A sudden increase in infection in estuarine and inshore waters is found to coincide with the offshore migration of the main stock. The infected fish apparently linger behind and may live for some time in the old habitats after the arrival of the next recruitment. The offshore migrants are seldom infected, and it is probable that infection does not take place in water deeper than about 5 fathoms.

The possible immediate causes of this 'lingering' phenomenon are examined, and two suggestions are made as to the nature of the physiological upset brought about by the presence of *L. branchialis* on these gadoids. Other factors influencing the distribution of the parasite are discussed.

The relative susceptibility of the different species of gadoids to infection with species of *Lernaeocera* is examined as far as the data allow.

The duration and distribution of the developmental phases on the intermediate pleuronectid and final gadoid hosts are estimated: the former being probably not more than two weeks, and the latter about six to eight weeks. Corroborative evidence for the length of the gadoid phase is obtained from certain epizootic organisms. The production of a vast number of eggs and their protracted shedding result in a scattered series of larval forms at all times of the year on the gills of flounders caught at the seaward end of the estuaries. There is likewise a scattered series of adult substages on gadoids. The numerical abundance of each phase is taken as being proportional to the length of time spent in each phase. There may be as many as five or six consecutive generations a year, but these generations show an infinite degree of overlap. The infection of the 0 year-class fish after their few weeks' sojourn in inshore and estuarine waters is correlated with the large numbers of larval forms found on flounders in this neighbourhood.

The lingering of parasitized gadoids, in effect, ensures the reinfection of the flounders: the ripe eggs from the female *Lernaeocera* on the gadoid are thus shed in shallow water where the conditions are optimum for the early development and for the minute free-swimming copepodids and their subsequent infection of the flounders on the gills of which they begin their metamorphosis. The coherence of these facts, and the rarity of infected gadoids in offshore habitats, suggest that *L. branchialis* is, *par excellence*, an inshore parasite. On the other hand, the relatively frequent occurrence of *Gadus luscus* infected with *Lernaeocera lusci* in offshore waters suggests that infection takes place there and that the intermediate host is a pleuronectid more adapted to deeper waters than *Pleuronectes flesus*. That this intermediate host can also live inshore is evidenced by the occurrence of occasional infected *Gadus luscus* among the young stock in the estuary during 1936–7.

Multiple infection with *Lernaeocera* of different ages, both on the flounder and on the gadoids, indicates that these fish do not develop an immunity to reinfection, and it may even be possible that infection and the consequent prolonging of inshore and estuarine life may favour reinfection. The distribution of the adult substages on *Gadus merlangus* and on *G. pollachius* is very similar in all respects.

The distribution of *Clavella uncinata* has been similarly studied on the two common gadoids from estuarine, inshore and offshore waters near Plymouth. *Clavella*, unlike *Lernaeocera*, is confined to a single host throughout its life cycle; it is therefore not subject to the same restrictions regarding breeding habits as the latter parasite, and successful egg production can take place at any time during the life of the host: it is independent of the migratory habits in this respect, though other limiting factors appear to be operative. As with *Lernaeocera*, infection is first recorded in the 0 year-class fish a few weeks after their inshore migration. In strictly inshore waters (Plymouth Sound) the rate of infection continues to increase with the age of the whiting and pollack, but in estuarine waters nearby the infection is at first moderate but later (after the period of maximum growth of the fish) tends to fall off: this may be an expression of inclement conditions for the infective stages, such as the reduced salinity of this habitat during the autumn and winter. The effect of reduced salinity on *Clavella* parasitizing the cod in Danish waters is also discussed. *Clavella* appears to be about equally common on the cod, whiting and pollack, but it is rare on *Gadus minutus* and it has not been found by us on *G. luscus*. The duration and distribution of the later developmental substages are estimated in the same way as for *Lernaeocera*, and it is concluded that all the adult substages are completed in a few weeks, and that the complete cycle is perhaps not longer than two months. Reproduction takes place throughout the year, though since conditions are more favourable at certain times the main recruitment to the *Clavella* stock takes place in the autumn in the sea near Plymouth. Multiple infections were also common with this parasite so that it induces no immunity in its host.

The direct effect on the host is discussed; as with *Lernaeocera*, there is no discernible difference in the condition factors of infected and uninfected fish. The difference in the habitat and food of *Clavella* on three species of gadoids is indicated.

The species of *Lepeophtheirus*, like other caligids, are similar in body form and organization to the first stages of *Clavella* and *Lernaeocera*, and owing to their minute size and probable lack of osmoregulatory powers are unable to withstand salinity changes of any magnitude in the external medium as, for instance, when the host migrates into brackish waters. *L. salmonis* is more resistent than most caligids. *L. pollachii* has not been found in fish from estuarine waters and only occasionally in those caught inshore, but it is fairly common in fish caught out at sea. Its occurrence is primarily limited, however, by mechanical factors, i.e. the size of the sublingual space of the pollack, which is its invariable habitat. *L. pectoralis* is apparently most highly sensitive to reduced salinity, for it is strictly marine in occurrence: though quite adequate samples of *Pleuronectes flesus* were examined from estuaries no infected fish were found.

Data on the distribution of *Lernaeenicus sprattae* and *L. encrasicola* from *Clupea sprattus* in the estuary are included for comparison with *Lernaeocera*; though this is a consistently rare parasite, the records show that older infected fish tend to linger with the newly recruited estuarine stock of the following season. The effect on the host is discussed.

Acknowledgements

We wish to express our indebtedness to the Director and Staff of the Marine Biological Association's Laboratory for all the facilities offered under the present difficult circumstances, and for their individual help so generously given at all times. One of us (N. G. S.) wishes to thank the University of London for the permission to occupy the University table at Plymouth for two years, and for the award of the Post-Graduate Research Studentship (1939–41) which has made these investigations possible.

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OBSERVATIONS ON THE BIONOMICS AND PHYSIOLOGY OF *TREBIUS CAUDATUS* AND *LERNAEOCERA BRANCHIALIS* (COPEPODA)

By N. G. Sproston[1] and P. H. T. Hartley

From the Plymouth Laboratory

(Text-figs. 1–5)

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THE HABITAT AND DISTRIBUTION OF *TREBIUS CAUDATUS*

Trebius caudatus Krøyer is a parasitic copepod belonging to the family Caligidae: a remarkably uniform group which is among the least modified by the parasitic habit. The body form is similar to that of *Caligus* and *Lepeophtheirus*, but unlike the former it lacks the pair of frontal suckers, and unlike both of these common genera the fourth legs are biramous in both sexes. The most obvious adaptation to the parasitic habit in this family is the roundish flattened carapace, which has a sucker-like action and prevents the animal from being washed off the surface of the fish on which it lives. This action is easily seen when caligids are transferred to a glass vessel: if an attempt is made to remove them, the previously swimming animal comes to rest and applies its carapace to the smooth surface with surprising tenacity. Another adaptation is the presence of strongly chitinized hooks on various cephalothoracic appendages.

All those who have worked with caligids remark on their peculiar habit of swimming to the edge of the sea water when placed in vessels in the laboratory. If left for a time they will swim out of the water and perish by becoming dry. We have seen this in several species and in no instance did they attempt to return to the water. This peculiar taxis is difficult to explain in relation to their normal habits.

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The life cycle of all caligids is a simple one, involving only one host. For the most part the species are host-specific; but some are found on hosts of closely related genera, and these parasites are notably those which are not confined to a particular habitat on the host, being found in various locations and on both surfaces in flat fish. The strictly host-specific ones, such as *Lepeophtheirus pollachii*, found under the tongue of the pollack, are usually confined to a special habitat. Even those showing host specificity to a less degree, such as *L. pectoralis* on various pleuronectids, very rarely migrate to fish of other species in a crowded aquarium. Fish heavily infected with caligids have been brought to the laboratory from the quay packed together in baskets, and in no instance have we found parasites characteristic of one species transferred to the adjacent fish of other species, though the parasites were alive and active and conditions were moist enough for them to move from fish to fish had there been any tendency to do so. An example of an exceptional degree of freedom is *Caligus rapax*. This very common parasite is found most frequently on gadoids, but it is also found on a large variety of other teleosts and even on elasmobranchs; moreover, it is not uncommonly met with in the plankton (Lebour, 1931, p. 173). *Trebius caudatus* has an intermediate degree of freedom: it is confined to elasmobranchs and is most common on Rajidae. Scott & Scott (1913, p. 82) mention that it is found '...on skates, rays, dog-fishes, etc.', but they do not give any specific names. It appears from the records that *T. caudatus* is confined to European waters, from which it is the only species of the genus recorded. Three other species have been found in the western hemisphere, also on elasmobranchs (Wilson, 1907, 1921). We have been able to add four new hosts to the records for this parasite, and three new hosts for the Plymouth district; the complete host list is given below.

Summary of Distribution of Trebius caudatus *according to Hosts*

Raja clavata Linn.
  Plymouth: Hartley, December 1934 (vide infra).
  Roscoff: Sproston, June 1937, on the upper and lower surfaces of the head, and rather more numerous (young, paler forms) in the gill cavities, spiracles and a few in the mouth.
R. maculata Mont.
  Plymouth: Oakley (in Leigh-Sharpe, 1933), 'on the dorsal surface'. Hartley, December 1934 (vide infra).
  North Sea area: cited by Lint & Stekhoven (1936).
R. microcellata Mont.
  Plymouth: Hartley, December 1934 (vide infra).
R. blanda Holt & Calderwood.
  Plymouth: Hartley, December 1934 (vide infra).
  Irish Sea: Great Orme to New Quay Head (Scott, 1929); Red Wharf Bay, N. Wales. Scott (1929) found seventeen females and three males on one fish.
  North Sea area: cited by Lint & Stekhoven (1936).
R. fullonica Linn.
  North Sea area: cited by Lint & Stekhoven (1936).

R. batis Linn.
  Polperro, Cornwall: Norman (1859) (in Norman & T. Scott, 1906).
  Plymouth: Bassett-Smith (1896), on the dorsal surface of the head and in the nasal cavities.
  Irish Sea: Belfast Bay, W. Thompson, September 1838 (in Baird, 1850). Great Orme to New Quay Head (Scott, 1929).
  North Sea area: cited by Lint & Stekhoven (1936), 'head and nasal cavities'.
R. marginata Lacepède.
  Roscoff: Sproston, June 1937, three medium-sized fish all had a few of these parasites, mostly on the underside of the head. One large fish had 18 adults on the upper and lower surfaces of the head, 10 young (pale) forms in the spiracles and 5 in the nasal cavities. None was found on medium and small fish examined in September–October 1938 at the same place.
Galeus vulgaris Fleming.
  Cited by Gerstaecker (in Bronn, 1866).
Acanthias vulgaris Risso.
  Roscoff: Sproston, September 1938, on the gills.

Note. The nomenclature of these fish is that used in the *Plymouth Marine Fauna*, 1931.

It is unfortunate that those studying parasitic copepods have seldom investigated their ecology, so that we have few records of the exact location of these animals on their hosts or other data concerning the host-parasite relationship. MacCallum (1916, p. 23) was one of the first to stress the importance of such observations when he drew attention to the nasal cavities of elasmobranchs as favourite habitats for ectoparasites—both for copepods and monogenetic trematodes—which habitat, he says, seems to have been overlooked. For instance, it was not until 1918 that Leigh-Sharpe first discovered *Lernaeopoda globosa* in the nasal cavities of *Scylliorhinus caniculus* (L.) Blainville, 1816; this parasite has since been found very frequently at Plymouth and at Roscoff, invariably in the same situation. Considering the large numbers of dog-fish examined annually in laboratories it is remarkable that it escaped notice until so recently.

Of the species of *Raja* examined by one of us (N. G. S.) at Roscoff in 1937 and 1938, it was noticed that most of the large fish were infested with *Trebius caudatus*, while only a few of the medium-sized fish and none of the small fish had them. They occurred on both surfaces of the head near the anterior end, and when immature forms were found they were invariably in the mucus of the nostrils, gill cavities and spiracles, and on two occasions a few in the mouth near the internal aperture of the spiracle. These immature forms were always pale in colour in contrast to the rather darkly pigmented adults. They were often associated in the mucus with the filamented eggs of *Rajonchocotyle* sp., a monogenetic trematode not uncommon on the gills. Of the twenty fish examined in detail at Roscoff, four, or 20 % (large to medium fish), were infected; this is about the same degree of infection as that found at Plymouth (see below).

During December 1934 one of us (P.H.T.H.) had the opportunity of examining several trawled species of *Raja* from the Mewstone Grounds and from Cawsand Bay, Plymouth. Special attention was given to the exact location on the host of the *Trebius caudatus* found, and other ecological data were noted. The distribution of these copepods on the different hosts from the two habitats is set out in Table I, and their distribution on the host is shown in Fig. 1.

TABLE I. THE DISTRIBUTION OF *TREBIUS CAUDATUS* ON *RAJA* FROM TWO LOCALITIES NEAR PLYMOUTH IN DECEMBER 1934

	R. blanda	*R. clavata*	*R. maculata*	*R. microcellata*	All species
Cawsand Bay					
No. of fish examined	28	13	20	9	70
No. of infected fish	2	1	5	2	10
Percentage infected	7·2	7·7	25	(22)	14·3
Mewstone Grounds					
No. of fish examined	0	14	7	1	22
No. of infected fish	0	5	3	0	8
Percentage infected	—	35·7	(43)	—	36·36
Both localities					
Total fish examined:					
Number	28	27	27	10	92
Length range: cm.	25–35	5–70	10–55	20–60	5–70
Mean length: cm.	23·0	30·3	29·1	37·5	28·5
Infected fish:					
Number	2	6	8	2	18
Percentage	7·2	22·2	29·6	20·0	19·6
Mean length: cm.	20·0	52·5	42·2	52·5	44·4

In spite of the small size of the samples, it may be permissible to point out certain items of interest in these observations at Plymouth in 1934.

The *Raja* caught on the Mewstone Grounds are between two and three times more heavily infected than those caught at the same time in Cawsand Bay. The depths of water in these two localities are respectively 10–15 and 3–5 fathoms, so that other things being equal, deep water appears to be more favourable to *Trebius caudatus* than shallow. One possible factor is that the parasite is less disturbed by currents in deeper waters. The fish themselves move but little, so that we feel justified in regarding the two habitats separately (see Steven, 1936).

On the whole, the larger the fish the more likely is it to be infected. No fish below 10 cm. long was found infected. The length distribution in three arbitrary groups in relation to the infection rates is given below (Table II). The arithmetic mean length of all the fish examined in 1934 was 28·5 cm. and that of the infected fish was 44·4 cm.

TABLE II. LENGTH DISTRIBUTION OF *RAJA* SPP.

Length group cm.	No. of fish examined	Infected fish	
		No.	%
5–25	48	2	4·15
25–50	34	11	29·5
50–70	9	6	66·6

The mean rate of infection of the species of *Raja* both at Roscoff and at Plymouth was about 20 %. The mean intensity of infection per fish at Plymouth was only 1·45 parasites per fish, but at Roscoff it was 6·0. The latter figure includes only the adult forms on the skin; the immature forms from the head cavities were not included. Unfortunately, these cavities and their contained mucus were not examined in the Plymouth samples, and no immature forms were found.

At Plymouth both sexes of fish were about equally parasitized: ten females and eight males.

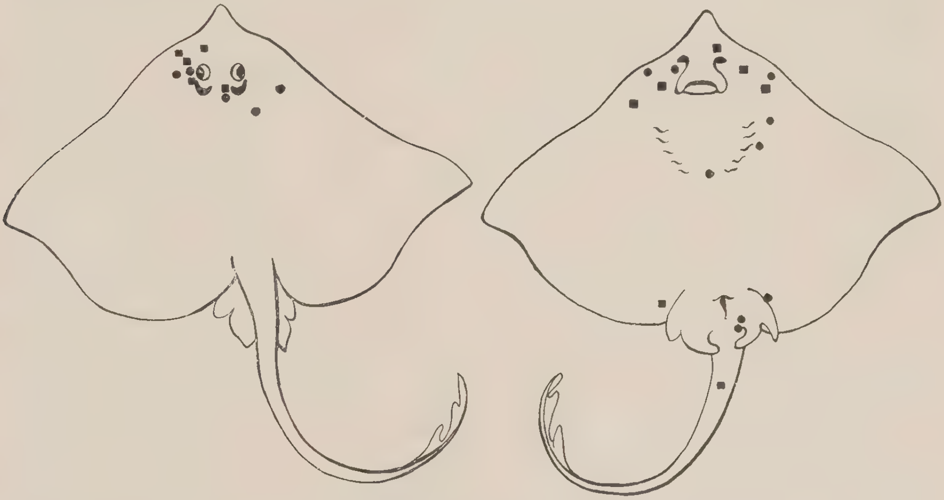

Fig. 1. Generalized diagram of *Raja* showing the location of each individual *Trebius caudatus* found in Dec. 1934. The black disks indicate males and the black squares females.

From Fig. 1 it is seen that the parasites tend to collect near the orifices on both surfaces of the body—ten on the upper side and sixteen on the under side—and that this distribution is also that of greatest concentration of mucus glands on the skin. It is understandable that this secretion would attract the parasites, for it is a necessary lubricant on so rough a surface and also is believed to form a large part of their food.

Of the *Trebius caudatus* collected at Plymouth, twelve were females and fourteen were males; the Roscoff records are not quite complete, but the proportion of the sexes was nearly equal, with a slight preponderance of females among the adults. This nearly equal distribution of the sexes is most unusual in caligids, in which the males are as a rule far less common than the females.

INTESTINAL RESPIRATION IN *TREBIUS CAUDATUS*

The cuticle of free-living copepods such as *Calanus* and *Cyclops* is very thin, and it is generally assumed to permit gaseous exchange, not only through the thin appendages but also over the whole surface of the body. In the larger copepods, however, the specific surface (i.e. surface per unit volume) decreases significantly and the cuticle becomes thicker to withstand the stress of stronger musculature, so that superficial respiration alone is evidently inadequate.

The term 'anal respiration' is generally applied to that phenomenon in various invertebrates whereby a respiratory current is taken in by the anus. In Copepoda it includes peristalsis of the gut (which is only partly concerned with respiration), various movements of the hind body as a whole and the actual gaseous exchange through the gut wall; at the same time these movements are transmitted to adjacent organs and the body fluid between them, so that they serve as the motive power for a two-way circulation. We prefer to use the term 'intestinal respiration', because it does not exclude the homologous phenomena in those parasitic copepods in which there is no anal current.

Various authors have described rhythmic gut movements (peristalsis and 'anti-peristalsis') in copepods, and in those lacking a heart such movements have been considered as supplying the motive power for general circulation of the body fluids. Claus was strongly opposed to Weismann's interpretation of these movements as respiratory in function, in spite of numerous authors' observations of a current of water entering and leaving by way of the anus, in copepods, and in other crustacea of the same order of size. According to Hartog (1888, p. 26), Claus's chief difficulty in accepting anal respiration as a normal function lay in his conception of the function of the rectal dilator muscles as solely concerned in defaecation. Hartog, in his monographic account of the morphology of *Cyclops* (1888), shows that it is these muscles which are primarily concerned in admitting and ejecting the respiratory current through the anus. He shows that their action, in series with those of the higher reaches of the gut on the one hand, and that of the circulatory disposed muscle fibres in the walls on the other, have the combined action necessary to effect anal respiration. The events are thus described by Hartog (p. 25): the anus is dilated when the gut is stretched to its maximum extent, which allows free ingress of water, the anal valves then close and the rectum is pulled forward: the stomach meanwhile begins to expand and becomes strongly arched so that the anterior region of the gut assumes a marked sigmoid curve in the antero-dorsal direction, which causes a forward movement of the water recently taken in. With the immediate flattening of the sigmoid curve the stream of water is reversed and flows again into the hind gut as this, in turn, straightens out, and makes its exit as soon as the anal valves open. A fresh stream of water enters at the recommencement of the cycle, just as the rectum is being drawn forward and immediately before the anal valves close.

Probably the fine teeth or coarse setae described by Hartog (1888, p. 24, Pl. 3, fig. 2) as occurring on each anal valve, serve to filter the respiratory current, though he does not offer this as an explanation. Apparently the valves in the intestine (also figured and described by Hartog) are too fine to allow the passage of food with the current, but he states (p. 26) that the rhythmic cycle is interrupted when a bolus of faeces collects in the rectum, but immediately on its expulsion movements are resumed with an extraordinary vigour.

This author claims to have observed a similar regular rhythmic anal respiratory current in three groups of free-living copepods and also in *Caligus* and in *Argulus* (as well as in cladocerans, gammarids, *Asellus*, larvae of *Apus* and *Zoea*). Wilson (1905, pp. 516–17) states that he has observed movements associated with anal respiration in *Lepeophtheirus edwardsi*, *Caligus rapax* and *C. bonito*, and that it was '...exactly like that described by Hartog for *Cyclops*, save that it was not as regular'. He does not, however, give any details of his observations. Scott (1901, p. 20) describes an intermittent two-way peristalsis in *Lepeophtheirus pectoralis* and a concomitant jerky two-way circulation of the blood; but though he describes anal dilator muscles in this species he remains sceptical of anal respiration. This scepticism is maintained by Scott & Scott (1913, p. 14).

We have both observed this phenomenon in various species of caligids from time to time, and we find that the underlying mechanism seems to be substantially the same as that described by Hartog for *Cyclops*, the action of the longitudinal muscles attached to the gut working in harmonic sequence, superimposed on the peristaltic movements of the gut itself. Here, however, the agreement ceases. Some years ago, one of us (P.H.T.H.), working at Plymouth, made an attempt to investigate the rhythmic nature of respiration in *Trebius caudatus*. It appeared far more complicated than that described by Hartog, and at first sight it seemed that the welter of pulsations was entirely arhythmic. The irregularity was apparent not only in numerous individuals on different occasions but in the same individual when examined over periods of 6–9 minutes. The main results of these observations on *Trebius* are summarized below:

(1) Peristaltic waves proceed along the entire length of the gut in both the forward and backward direction.

(2) In regard to these movements the three sections of the gut, the cephalothoracic, that in the genital segment and that in the abdomen, appear more or less isolated, and each has its own set of peristalses. These may, or may not, be transmitted to the adjacent segments.

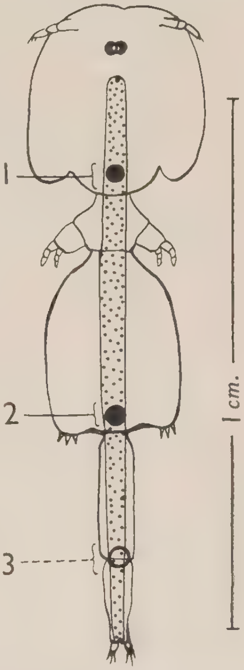

Fig. 2. *Trebius caudatus* (female) (diagrammatic), showing gut and the three centres controlling peristalsis.

(3) The peristaltic waves appear to be initiated from three centres which are situated towards the posterior end of each gut section: these are shown dia-

grammatically in Fig. 2. The exact situation of the abdominal centre was difficult to determine and its approximate position is indicated by an open circle.

(4) Each section of the gut is able to initiate contractions in either direction, but at times a wave of contraction started in one section may be continued into another. Forward peristalses in the abdominal gut, the only type of continuation observed with any frequency, were often continued into the genital gut. Backward peristalses of the genital gut were twice seen to be continued into the abdominal section. In one set of observations only, backward peristalses in the cephalothorax were seen to be continued into the genital section on three occasions during 6 minutes.

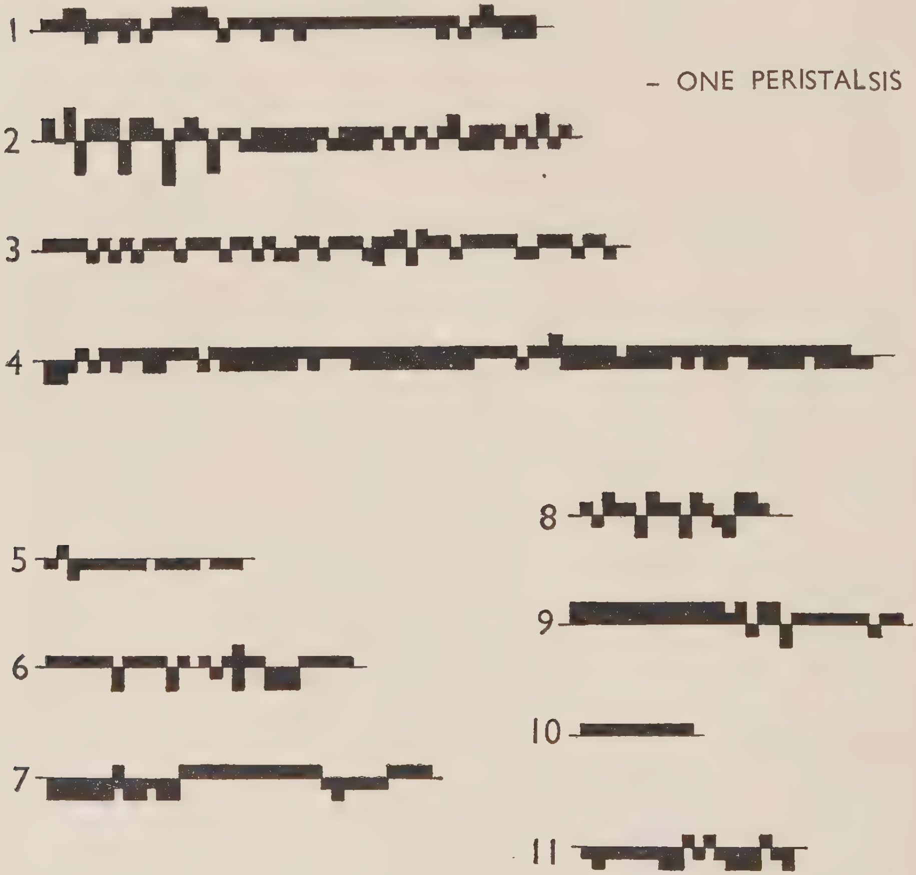

Fig. 3. Graphical representation of the direction and relative intensity of the peristalses in *Trebius*: 1–4 from the cephalothoracic section of the gut, 5–7 from the genital segment, and 8–11 from the abdominal gut. The records are of selected typical examples, and the blocks above and below the central line represent forward and backward peristalses respectively.

(5) Peristaltic contractions were more numerous per unit time in the cephalothoracic than in the other two sections.

(6) Simultaneous peristalses in both directions were characteristic of the cephalothoracic section. They were very infrequent in the genital section (1·5 % of the total observations only), and unknown in the abdominal section.

(7) The duration of peristalsis in either direction was extremely variable, as was the intensity (strength of beat) and the frequency of the waves of contraction.

(8) It is beyond doubt, however, that forward peristalses were the more numerous.

Fig. 3 illustrates the change of direction and the relative intensity of some selected typical series of peristalses in each section of the gut. The value of the time axis is not given. These particular examples show extreme irregularity in the maintenance of peristalsis in a given direction; the relative intensity of the beats is also apparently capricious. Table III gives the means of the frequencies of peristalses in the complete series of observations.

TABLE III. THE FREQUENCY OF PERISTALSES IN *TREBIUS CAUDATUS* (PER MIN.)

	Total peristalses			Backward peristalses only		
	Cephalothorax	Genital segment	Abdomen	Cephalothorax	Genital segment	Abdomen
	6·85	1·40	5·00	1·57	0·80	0·5
	4·36	0·60	1·28	0·535	0·20	0
	4·84	1·00	1·66	0·526	1·00	0
	11·00	3·86	9·5	3·67	1·17	0
	12·00	1·50	0·50	2·33	3·00	0
	9·00	5·83	3·16	7·33	0	2·67
	12·16	2·33	—	4·00	—	—
	5·00	7·00	—	8·83	—	—
Arithmetic means	8·15	2·94	3·52	3·60	1·03	0·53
Means of forward peristalses				4·55	1·91	2·99
Ratio of forward/total peristalses (%)				55·8	65·0	85·0

These observations were made under approximately similar laboratory conditions, and though the temperature factor was not controlled (albeit highly important), the high degree of variation observed during the short periods of observation cannot be due to this variable: it is probably a normal attribute of the process. Owing to this high degree of scatter, the number of observations is insufficient for the results to be treated statistically, so the arithmetic means alone have been reproduced in the table.

It is instructive to note that the frequencies of the forward peristalses expressed as percentages of the frequencies of the total peristalses (when the means are employed) increase rapidly from anterior to posterior ends of the gut. This—the increasing residuum of forward over backward peristalses, from head to tail—is one of the most significant features of that type of intestinal respiration which involves an anal current, for it ensures that the contents of the gut are retained. It would seem that there is a necessary fall in the pressure gradient along the gut towards the anterior end, and that this prevents too great a movement in the middle region of the intestine where digestion is believed to take place; it would also reduce the chances of regurgitation through the mouth to a minimum. Much of the irregularity of the gut movements may be due to the impedance caused by the presence of more or less bulky food and faecal matter.

It was not possible at the time to make simultaneous observations on the relation of the peristaltic movements as a whole to the actual intake of water by the anus. A gaping of the anal lips followed by a closure strongly suggestive of the intake of water was seen repeatedly in *Trebius*; and in *Paracalanus* sp. the uptake of beads of water was seen distinctly on several occasions. In *Trebius* there was also a definite rubbing together of the anal lips: they showed, immediately after the intake of water, a differential up and down movement—along the antero-posterior axis—as if one side contracted and then the other. Lastly, it may be mentioned that the intake of water was always accompanied by a brisk wagging of the abdomen as a whole, a movement so violent that often it affected the whole body. This up and down beating of the hind body is especially characteristic of the chalimus stages of parasitic copepods, and is always very obvious in the 'cyclopoid' and 'pupal' stages of *Lernaeocera branchialis* on the gills of the flounder: this species is dealt with below.

The highest frequency of total peristaltic beats occurs in the cephalothorax (Table III): this may well have a digestive rather than a respiratory significance, for simultaneous amphidirectional peristalsis was the general rule in this region, and would help the trituration process in the stomach. The amphidirectional peristalsis took place by a wave of contraction starting forwards from centre 1 (Fig. 2) which was balanced by a posterior wave starting, apparently at the same time, from the anterior limit of the gut: it had, in fact, the appearance of being reflected from the anterior wall. The observed effect was a regular wave, the wave-length of which was equal to the length of this section of the gut. If another initiation centre is present at the anterior limit of the gut, it is necessarily only concerned with backward peristalses.

The subdivision of the gut into three sections is a further aid to the retention of food in the respective regions for a sufficient time for digestion to be completed. Scott (1901, p. 16) has shown that there are no valves separating the sections, but Wilson (1905, p. 510) points out that there is a constriction at the junctions of these regions '...like the beginning of a sphincter muscle, but the opening cannot be closed'. Hartog has, however, demonstrated valves in *Cyclops*. Another safeguard against the premature passage of food from the stomach is the normal discontinuance of the cephalothoracic peristaltic waves into the genital segment. The rare instances in which this was seen may mark the passage of food through the sphincter-like constriction, and so be part of normal alimentation.

The continuation forwards of peristaltic waves from the abdominal into the genital gut, on the other hand, is part of the respiratory movements and is necessarily the rule in this section of the gut: handing on, as it were, the respiratory current from the rectum to the intestine.

The vigorous action of the longitudinal muscles moving the water current from stage to stage along the gut is doubtless the cause of the movements of the hind body as a whole. This is naturally more conspicuous in so light an animal as a chalimus, suspended as it is by its frontal filament: the muscular contractions cause it to swing through a considerable arc. The peristaltic movements serve to stir the water (oxygen vector) in each section of the gut and so effect maximum efficiency in the gaseous exchange through the walls. The generalized peristaltic movements are transmitted to the blood in the surrounding haemocoele and account for the discontinuity in blood circulation already referred to, and observed by Scott (1901, p. 20) and Wilson (1905, p. 515) as well as by many earlier authors.

The respiratory movements in caligid copepods show, therefore, an irregular regularity.

The Relation of the Internal and External Environment to the Feeding of *Lernaeocera branchialis*

Lernaeocera branchialis (Linn.) presents a complete contrast to the caligids in almost every respect: it is one of the most highly modified of the parasitic copepods. The adult female is permanently fixed to a gadoid with its head in the vascular tissue in the region of the heart; its food is almost entirely the blood of the host; its cuticle is relatively highly chitinized, so that it is more independent of its environment; and its life history is complicated by the interpolation of an intermediate host which the male never leaves. The first copepodid stage is free swimming as in *Trebius* and other caligids, and in general body form it is not unlike them. This stage finally settles on the gills of a flounder (*Pleuronectes flesus*) where it undergoes part of its metamorphosis (see Sproston, 1941), resulting in a free swimming cyclopoid male, and a female which is similar except for an elongated genital segment. Temporary attachment of these latter forms is effected by the chelate second antennae. After pairing, the male may return to the gill filaments, though it does not live for long; but the female becomes pelagic and seeks certain gadoid fish on which the metamorphosis is completed.

The following observations on *Lernaeocera branchialis* were undertaken by one of us (N. G. S.) at Plymouth during the period August 1939 to November 1940. It was mentioned above that respiratory movements were observed in the developmental forms on the flounder, and was accompanied by vigorous motion of the body as a whole which was visible to the naked eye. Detailed observations were only made on the adult female; although peristalsis in the thinner part of the hypertrophied genital segment could be seen while the parasite was in situ, no details could be made out until it was excised and put in a strong transmitted light under a low power microscope. The parasites were accordingly carefully dissected away from the tissues of the host and placed for a time in circulating sea water, in which they remained apparently healthy for periods up to 9 days. The rate and vigour of the peristalsis did not appear to have diminished and it was assumed that the animal had not suffered any marked derangement in its functions by removal from the host. Important consequences of this, however, would include the gradual starvation and changes in osmotic equilibrium consequent on the change in internal environment, viz. the gradual substitution of sea water for gadoid blood in the intestine. These suppositions are supported by direct measurements of the osmotic pressure of the body fluid of the adult female *Lernaeocera* (Panikkar & Sproston, 1941).

It was found that the adult female *Lernaeocera*, unlike most marine invertebrates, is able to maintain a body fluid hypotonic to its external medium so long as it is attached to the host. It presents a complex osmotic system by virtue of its feeding on hypotonic blood, on the one hand (that of *Gadus pollachius* having an osmotic pressure equivalent to 1·443 % NaCl), and by being bathed externally by sea water on the other, the body fluid of *Lernaeocera* having an osmotic pressure equivalent to 2·0–2·8 % NaCl. When the parasite is removed from the host, however, isotonicity is established with the external medium owing to water being taken in through the mouth in place of the blood of the host. *Lernaeocera* was nevertheless still able to tolerate dilution of the external medium up to 2·243 % NaCl, which represents a salinity probably lower than that normally tolerated by the host.

Since the above paper was written other experiments have been conducted (by N.G.S.) which show that isolated *Lernaeocera* is unable to survive 50 % sea water for much longer than an hour. On three occasions perfectly healthy *Lernaeocera* were transferred to normal sea water after removal from the host, and when normal peristalsis had been verified they were subjected to a gradual dilution of the external medium and left in approximately 50 % sea water. They all became pale and peristalsis was increasingly disturbed and weakened, finally ceasing altogether; by this time the blood in the gut had been lost through the mouth (the actual stream of blood seen in one specimen only). Death, as judged by cessation of peristalsis, and complete pallor occurred in about an hour in two examples and in a little longer period in a third. The physiological effect was probably that of a violent endosmosis of the now hypotonic medium into the tissues of the parasite most probably via the alimentary canal—some vacuolation was seen in the body tissues of one specimen.

Since in the natural condition on the host a marked hypotonicity is maintained by the body fluid, any intake of water by the anus is precluded; indeed, the anus was carefully observed continuously for periods up to 20 min. (the period including several changes in direction of peristalsis), but no ejection of fluid was seen or any movement in this region which could suggest that the anus was functional. To verify this, fine particles in suspension were watched in the neighbourhood of the anus but no movements occurred suggestive of an anal current.

The abdomen of this species has been figured in section by Scott (1901, Pl. V, fig. 4), in which the anus is shown as an aperture, but neither he nor subsequent authors actually mention that it is functional.[1] From the physiological considerations elaborated above we consider it non-functional, and examinations on the living animal do not disprove this. On microscopical examination of the whole parasite, a flattened funnel-like depression is seen at the posterior extremity, but it does not perforate the cuticle, and the rectum apparently ends blindly. It is not surprising that *Lernaeocera* has become aproctous in its final adult stage, for like many blood-feeding parasites the digestion is slow but complete.

No movement of fine particles in suspension in the neighbourhood of the mouth was observed in living (isolated) *Lernaeocera*, and it was assumed that this aperture was also closed during the period of observations. Though the mouth is morphologically of the suctorial type, we do not consider that it is functional, as such, in the adult female; for suction would certainly be superfluous against the pressure of the blood stream of the fish. Furthermore, the histological changes taking place as the result of traumatic injury by the parasite, as recently shown by Stekhoven (1936), consisting of a fibrotic thickening of the ventral aorta and bulbus arteriosus, produce a stenosis of the lumen in this region and a consequent increase in the blood pressure. The difficulty for the parasite, would, it seems, be the exclusion of an inrush of blood through its mouth. In view of these considerations it is not surprising to find that the mouth is normally closed, probably by valves, and that it opens only at well-spaced intervals to admit a meal of blood which is retained in the gut for a long period. In support of this it may be mentioned that the isolated specimen which survived 9 days in circulating sea water showed only a slight fading of the original red colour of the gut at the end of this time.

The actual feeding depends, in our opinion, on the maintenance of a dynamic equilibrium of the osmotic system referred to above, and involves the following set of factors. A slow osmosis occurs through the body wall, i.e. water will pass out owing to the tendency for isotonicity to be established, and if salts enter they must do so far more slowly than water escapes. The gradual loss of water in this way would finally reduce the hydrostatic pressure in the gut of *Lernaeocera* to a limiting value which would determine the intake of a fresh supply of blood through the mouth: that is, the internal pressure in the gut gives way to the blood pressure of the host. This discontinuous feeding is supported by the findings of Panikkar & Sproston (1941, Table II) on the osmotic pressure of *Lernaeocera* in situ, where a considerable variation in values was observed for the body fluid: the low values would represent a comparatively recent meal of the hypotonic blood of the host, and the higher a corresponding degree of starvation—the body fluids having been concentrated by gradual loss of water through the cuticle.

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[1] Brady (1880) quotes Krøyer's observation that on touching the body of a mature female *Lernaeocera* a jet of liquid was forcibly ejected from the hind end of the abdomen. No one has been able to confirm this observation. We suggest that the animal was inadvertently punctured and that the contents, being under pressure, would, and do, escape in the form of a jet.

Microscopical examinations made on the contents of the gut (by withdrawing it through fine glass cannulae as described in the paper cited) while the parasite was still attached to the host, and some 2 hr. after the death of the latter, showed a few fish erythrocytes which were quite normal in appearance and had no signs of crenation or other effects of digestion. The body fluid was examined in a similar way: it was reddish yellow in colour and contained even fewer cells; these were minute stellate cells which rapidly disintegrated, and were considered to be thigmocytes (proper to the blood of Crustacea). Stekhoven has made similar investigations (1936) and he found no fish erythrocytes in the body fluid; he considers that the red colour of the latter is due to a specific respiratory pigment which may, he thinks, be a 'reconstructed' pigment from the blood of the host, dissolved in the body fluid of the copepod, and acting as an oxygen carrier. He maintains that it is distinct from haemoglobin on the grounds of different colour; but he admits that he has not submitted it to micro-spectrographic tests. It is regrettable that our material was insufficient for such tests. In our opinion the different colour (reddish yellow) of the body fluid is due to its own intrinsic yellowish colour partly masking the haemoglobin, and that this yellowish tinge is partly the result of digestion, for fat droplets were frequently seen in suspension which were deep yellow in colour. We should expect to find that the reddish tinge was due to haemoglobin in solution, this having passed through the gut wall after the breakdown of the erythrocytes and accumulated in the body fluid as the parasite grew older. The more advanced the *Lernaeocera* the deeper we found its coloration to be: those females with partly spent egg strings were deep red, and those with spent egg strings were purplish red and the abdomen almost black. When the excised parasite was placed in circulating sea water the colour faded but little, except on two occasions when the specimens died prematurely. In these specimens the gut lost the red colour on the third day and on the succeeding day the body became increasingly pale until it died: the final colour on death was a brownish yellow.

Vanden Berghe (1933) made some investigations on the alimentary canal of *Lernaeocera*. In his discussion of the subject he rightly dismisses the remarks of Hesse (1863) on the subject of feeding in *Lernaeocera* as being confused and fantastic. Hesse also 'describes' (without figures) a heart and blood vessels which Vanden Berghe very justly denies, though his further remarks to the effect that both heart and blood vessels are unknown in

Copepoda are not accurate, for a saccular heart is present in Calanidae and Pontellidae and Ed. Van Beneden demonstrated blood vessels in *Lernanthropus* (also in *Congericola* and *Hatschekia*), and proved that they contained haemoglobin in solution (see his claim to the priority for these discoveries, 1880); the complex vascular system in *Lernanthropus* was later described by Heider (1879) in his monograph on the subject, and, in American species, by Wilson (1922).

Vanden Berghe examined a large number of *Lernaeocera* from *Gadus morrhua* and *G. luscus* at Wimereux during the summers of 1931 and 1932, but his findings differ curiously from those of Scott (1901), Stekhoven, Punt (1936, 1937), and our own. He says that the red colour resides entirely in the haemocoele and that the gut of all his specimens was devoid of red colour. He examined the fluids by making an incision across one of the antlers and states that the haemocoele fluid which issued was of a bright red colour and labile; but on making a deeper incision into the body the liquid from the gut was liberated, which was viscous and yellow in colour. We can only assume that during the difficult process of dissecting out the parasite from the host tissues, some unnoticed injury occurred, perhaps to the proboscis, that the original contents of the gut escaped before the above examinations were made, and that the liquid he observed coming from the gut was merely the residuum of the original contents mixed with the tissue fluids. From the absence of all but traces of fish blood in the gut, and because he was unable to extract an anticoagulant from any part of the alimentary system, he concluded that meals are taken at well-spaced intervals and rapidly digested. We, on the other hand, while agreeing that meals are rare, consider digestion to be a very slow process for, as explained above, by the micro-dissection methods employed we had no difficulty in extracting red liquid from the gut which was certainly no more viscous than the haemocoele liquid. It is difficult to see how Vanden Berghe reconciled these conclusions with his other findings on the rapid propagation of peristaltic waves in the intestine. In our experience the peristalses were conspicuous; for as the waves of compression and rarefaction travelled along the gut its red colour showed up as a band of colour of varying intensity against a background of a less intense red, which was tinged with yellow in the younger specimens.

Intestinal Respiration in *LERNAEOCERA BRANCHIALIS*

Observations were made on the peristaltic movements of *Lernaeocera branchialis* both from the whiting (*Gadus merlangus*) and from the pollack (*G. pollachius*); the length of continuous observations was between 20 and 40 min., but on two occasions it exceeded an hour. These two long records are reproduced in graphical form in Fig. 4 together with two others. Fig. 4 is in no way comparable with Fig. 3, for in it the abscissae represent the time in minutes during which the animal was observed and the ordinates, the number of peristalses per minute passing through the mid-point of the genital segment; both forward and backward peristalses are recorded on either side of the abscissa passing through the origin. *a* is a recording from a freshly isolated parasite from the pollack: A–B was taken after the parasite had been in circulating sea water for only 2 hr., and C–D after the same specimen had remained in circulation for 48 hr., and it is seen that there is little difference in the type of recording. In both the backward wave was accompanied by a forward one and these latter waves continued throughout the period of observation. This simultaneous, amphidirectional, peristalsis was rather unusual in our specimens. *b* is a record from a parasite removed from *Gadus merlangus* after it had remained in sea water for 24 hr., and *c* is one from another specimen from the same species of host on another occasion; in neither is there an amphidirectional peristalsis for more than a few beats at a time and this only occurred at the change-over from the backward to forward direction. In the change from forward to backward there was sometimes a slight pause (never more than half a minute, and usually less) at the actual change-over.

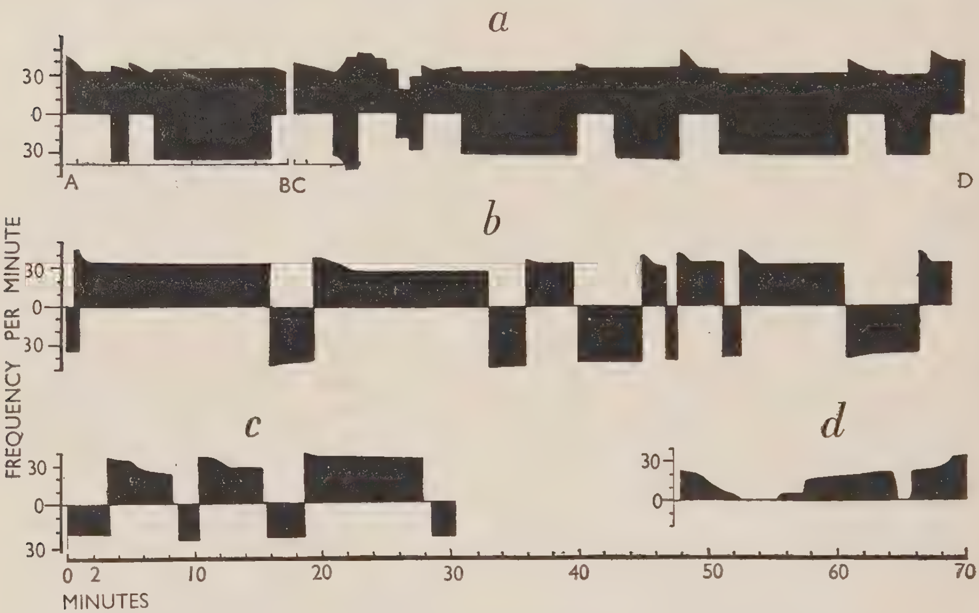

Fig. 4. Graphical records showing the frequency and direction of peristalsis in the genital segment of adult female *Lernaeocera branchialis*. Anterior and posterior peristalses shown respectively above and below the central line; frequency of waves per minute represented along ordinates and time in minutes on the abscissae. *a*, specimen from *Gadus pollachius*—A–B, 2 hr. after isolation of parasite from host; C–D, same specimen 48 hr. after isolation. *b*, specimen from *G. merlangus* 24 hr. after isolation. *c*, another specimen from same host 24 hr. after isolation. *d*, specimen from *G. pollachius* which had remained in situ on dead host for 7 days at 2° C.; recorded after specimen had been isolated and had remained in sea water for 3½ hr. at room temperature.

In each of these records (as in others not reproduced here) the initial forward contractions were stronger and more frequent in unit time than the subsequent ones: they started off at high speed which rapidly diminished, reaching a normal value within 1·5–2 min., and this frequency was then maintained steadily till the end of the phase. Only on two occasions did the backward peristalses start with a higher rate of contraction than the normal, and on a few they started at a lower rate which was soon brought up to normal.

As in *Trebius*, there appeared three initiation centres for the peristaltic waves in *Lernaeocera* (these were mapped out by Sproston before Hartley's observations were made known to her) and it is of great interest to note that their positions are homologous in two such widely differing copepods. Fig. 5 is a diagrammatic optical section of *L. branchialis* in which the approximate positions of these centres are indicated. Some attempt has been made to show the relative thickness of the cuticle in different regions of the body wall by the thickness of the outline: that of the antler-like outgrowths of the cephalothorax is very thick indeed, so that there is little space within them; elsewhere, particularly in the posterior two-thirds of the genital segment, there appeared a wide area of haemocoele (notably in advanced females) and at times some movement could be seen therein as a direct consequence of the peristalsis; but no movement, i.e. circulation, could be made out in the cephalothoracic region. The abdomen is also strongly chitinized and this made observations of peristalsis very difficult except in young females.

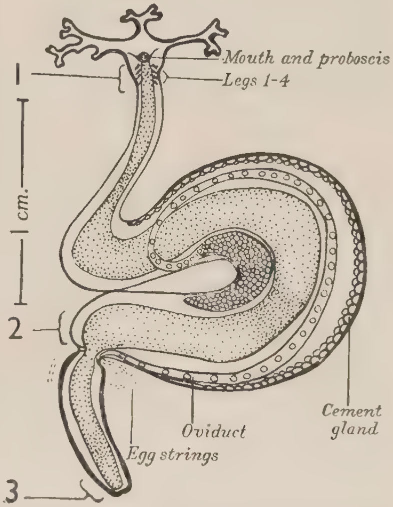

Fig. 5. *Lernaeocera branchialis* (adult female) (diagrammatic), showing the regions from which peristalses arise. These three 'centres' are homologous with those of Fig. 2.

The comparatively few observations we have on the abdominal peristalses agree in that they seldom coincide with those in the genital segment. Forward peristalses were less frequent in unit time than those in the genital segment so that they appeared out of phase, and they were never continued forwards past centre 2 into the genital segment; their frequency was fairly constant—from 16 to 20 per minute—but their intensity was feeble. Backward peristalses were also less frequent than the corresponding waves in the genital segment; they were fitful and sometimes the impulses failed to be transmitted; they appeared to originate from centre 2 and not be merely 'handed on' from those of the genital segment which originated from centre 1. Other instances of impedance were met with in the genital gut immediately on the cessation of a set of forward peristalses, when there was a pause before the impulses from centre 1, in the hind region of the cephalothorax, were manifested as backward waves (Fig. 4 *b*, *c*). On the other hand, immediately on the cessation of the impulses from centre 1, centre 2 appeared to be relieved of some depressor action and the forward impulses were abnormally strong and frequent (Fig. 4 *a*, *b*, *c*). On rare occasions the depressor action was insufficient to quell the normal impulses from centre 1, and they continued concurrently with those from centre 2, resulting in the amphidirectional peristalsis observed throughout this specimen (Fig. 4*a*).

A further demonstration of impedance in centre 1 was found when a specimen of *Lernaeocera branchialis* was kept in situ on a pollack in the refrigerator at 2° C. for 7 days. On removal at the end of this period the parasite was opaque and no movement was visible within it; it was dissected out and placed in circulating sea water at laboratory temperature, in which it became translucent and peristalsis was resumed—very fitfully at first, but after 3 hr. it became stronger and more or less continuous but in the forward direction only. The recording after 3½ hr. is shown in Fig. 4 *d*. No backward waves were seen. After 24 hr. the forward peristalses were still in progress, with pauses of 1–5 minutes; the animal was then sacrificed for osmotic pressure determinations (Panikkar & Sproston, 1941, Table II (5)).

Stekhoven & Punt (1937) investigated the temperature effects on the peristalsis in *Lernaeocera branchialis* from the cod; they found that lowering the temperature reduced the frequency of the peristalsis, and also that the backward peristalses were almost completely inhibited by reducing the temperature to 5·5° C. for short periods (about 15 min. only in their experiments), but resumed their normal frequency when the temperature was raised again. They also determined the duration and frequency, under normal laboratory conditions, of the peristalses of seven specimens of *L. branchialis* and of two specimens of *L. lusci* (which gave substantially the same results). We have summarized their data and calculated the arithmetic means for comparison with our own (Table IV).

Table IV. The Duration and Frequency of the Anterior and Posterior Peristalses in the Genital Segment of *Lernaeocera branchialis*

Summary of Results obtained by Stekhoven (1936), *Stekhoven & Punt* (1937) *and Sproston* (*present paper*)

	Forward		Backward	
	Duration min.	Frequency per min.	Duration min.	Frequency per min.
Stekhoven: Stekhoven and Punt				
Arithmetic means	2·75	44·0	2·12	37·5
Range	0·6–7·8	25·0–72·6	0·58–3·75	22·5–60·5
Sproston				
Arithmetic means	5·7	33·2	3·3	30·6
Range	2·0–15·4	19·0–48·0	1·0–15·0	19·0–48·0

From the preceding summary in Table IV and from Fig. 4 it is seen, without going into a statistical analysis, that our values vary over a wide range, but an approximate idea of the duration and frequency of peristalsis in both directions is given by the arithmetic means of our observations. The mean duration of backward peristalsis is just over half (58 %) that in the forward direction in our specimens, but the mean frequency per minute is only slightly less. The corresponding results obtained on the same species of parasite from the cod by Stekhoven (1936) and by Stekhoven & Punt (1937) show similar relative differences; but the durations of peristalses in a given direction were always much shorter than those obtained by us, i.e. about half, when the means of each set of observations are calculated. The frequencies of the peristalses in their specimens are, however, slightly higher than in ours. Considering the wide variations in both sets of results, we do not feel justified in analysing them further.

The conclusions arrived at by Stekhoven (1936) and Stekhoven & Punt (1937) are that there are two automatic centres controlling the gut movements of *Lernaeocera branchialis*: one associated with the circumoesophageal commissure and the other near the end of the mid-gut (also referred to as the caudal centre). The exact location of these centres is, of course, hypothetical; but it is curious that these authors do not refer to a centre in the abdomen, nor do they record the, admittedly weaker, series of peristalses in this region. In their analysis of the peristalses in the genital segment they state that the impulses from the hinder centre (centre 2 in our figure) have a greater frequency, are stronger and are also maintained for a longer time than those originating from the cephalic centre. Our observations also support this conclusion. Now in the change-over in peristalsis from one direction to another, Stekhoven mentions an overlap of waves; he implies that this normally takes place at each change-over, but that it is of a shorter duration in the backward-forward change-over. He did not record any pauses in peristalsis. On the other hand, we observed pauses occasionally, but they only occurred at the forward-backward change-over. Overlap of waves from both directions, i.e. amphidirectional peristalsis, was also occasionally seen by us, but only during the backward-forward change-over (Fig. 4). Though these details are slightly at variance with the results obtained by Stekhoven & Punt, their implication is the same: namely, that the forward impulses (those from centre 2) in the genital segment are in every way more effective, and that there is a lag or impedance imposed upon the backward impulses (coming from centre 1).

As Stekhoven & Punt overlooked Vanden Berghe's paper it is necessary to review some of the interesting results obtained by this author on the peristaltic movements of *Lernaeocera*. Vanden Berghe finds that the initial contractions in each set of waves are stronger and more frequent than the succeeding ones (a point not mentioned by Stekhoven & Punt); they begin at about 44 per min. and gradually diminish, and the average duration for the backward phase is about 3 min., but that in the reverse direction it is about twice as long. This agrees very well with our findings (Fig. 4). He also mentions amphidirectional waves—disordered surgings to and fro—lasting for 2 or 3 sec. occurring at the backward-forward change-over, though he does not state exactly what happens at the other change-over. Like Stekhoven, he does not record pauses between the phases. He publishes very few actual records, and those, only of the number of waves of an entire phase of peristalsis. The means of these ten observations are: backward phases 65 and forward phases 107; he emphasizes this marked dissimilarity but he has found that it is far less marked in the younger specimens which had periods of the order of 50 and 70 respectively. We have not yet been able to confirm this.

Like Stekhoven & Punt, Vanden Berghe only recognizes two initiation centres: one near the mouth and one at the other extremity of the body, but unfortunately his statements are not very clear in regard to them, and not once does he mention that he noticed waves originating from the hind end of the genital segment. This is to be regretted for he goes on to describe some very interesting experiments in which he ligatured the body of a young specimen, first in one place and then in two places along the body. In the absence of diagrams the value of these experiments is diminished, for we do not know the position of the ligature in relation to the centre 2 in our figure. When one ligature was used an alternating peristalsis was seen as before, but the waves 'originating' from the ligature in both directions were weak and markedly less numerous than those starting from either of the ends. When two ligatures were used the central region between the ligatures showed no sensible movement. After consultation with V. Willem, Vanden Berghe concludes that, on a myogenic theory, these movements need not involve a special nervous intervention; for as peristaltic contraction is an intrinsic property of the gut wall, the reversal is explicable on the basis of fatigue and that the return wave at the single ligature is a reflected one.

We cannot wholly subscribe to this explanation, for it does not account for the difference in character of the peristalses in the genital segment and in the abdomen, nor does it cover the initial 'kick' in the forward waves, nor again the reduced facility of propagation of the backward series and their final suppression when submitted to low temperatures. We therefore maintain that it is probable that the intrinsic contractility of the muscle fibres in the gut wall is controlled by at least three autonomic nerve centres in copepods, and that the depressor action of centre 1 is significant in retaining the contents of the gut in those forms which utilize an anal current in respiration.

The Evolution of the Respiratory Mechanism in Parasitic Copepods

It is of interest, in passing, to review briefly the evolution of the respiratory mechanism in parasitic Copepoda. In *Trebius* and other caligids as well as in the developmental stages of the Lernaeoceridae an anal current introduces the water of the external environment which acts as the oxygen vector.

Hartog (1879) considered respiration in *Cyclops* to be 'entirely anal'. The gut movements are subservient to the circulation of a colourless blood in the lacunar system of the haemocoele. In the Dichelesthiidae, in such forms as *Congericola*, *Hatschekia* and *Lernanthropus*, there is a peculiar development of vessels containing blood with haemoglobin in solution, and this oxygen carrier supplements the respiration which takes place by means of an anal current and a lacunar system in the haemocoele. In *Lernanthropus* there are foliose outgrowths of the cuticle along the margins of which the large vessels run, and these are connected by a network in the substance of these appendages. The haemoglobin is probably obtained by diffusion through the gut wall in these blood-feeding forms. In the Lernaeoceridae an evolutionary series can be made out which shows a decreasing dependence on the anal current, or in other words, a tendency to become independent of the external environment. In *Lernaeenicus* the long thin body of the adult female hangs quite freely in the water and it is possible that cuticular respiration is of importance in this genus; it is probably not a blood feeder, but is nourished largely by the coelomic and tissue fluids of the clupeoid host. *Pennella*, though deeply imbedded in the skin of Cetacea, is not red in colour, and probably feeds on tissue fluids only. That it is still largely dependent on its external medium is shown by the presence of delicate feathery caudal appendages which are thought to aid cuticular respiration; also it is well known that *Pennella* is killed when the whales migrate into the colder polar waters. In *Peroderma* the short body is largely imbedded in the muscles of the back of the sardine and the head bears a tuft of thin-walled digitiform 'rhizoids'. This parasite has been the subject of numerous papers by Monterosso (1921–30), who shows that though the alimentary canal is still functional (he does not state whether the genus is aproctous), the cephalic rhizoids serve as absorptive organs both of nutriment in solution and of oxygen from the blood of the host. The rhizoids, he maintains, are syncytial in structure as are all the other organs of the body (Caullery (1926, p. 19) doubts this, and thinks that the syncytial appearance may be due to defects in fixation) and their growth in the renal tissue of the fish causes vascular hypertrophy, so that the rhizoids are bathed in host blood in the lacunae in which they come to lie. The haemoglobin which either passes into the haemocoele via the rhizoids, or via the gut wall, or both, acts as an accessory vector of oxygen; there may or may not be an anal current, but active peristalsis is noted in the gut, especially in the median section, and waves are said to be initiated in the posterior region. He emphasizes the kinetic function of the gut in relation to the body fluids.

In *Lernaeocera* the cuticle has become particularly heavily chitinized and the cephalothoracic outgrowths are purely mechanical anchors; cuticular respiration is reduced to a minimum and both oxygen and food enter the animal through the mouth in the form of the blood of the gadoid. A vigorous two-way peristalsis keeps the ingested blood in circulation, so that absorption of solutes is facilitated, and at the same time a feeble circulation is transmitted to the haemocoele fluid. Independence of the external environment has reached a high level as shown by the osmotic gradient and by the toleration of brackish water by this genus of parasites.

A comparison of the details of respiration in *Lernaeocera* and in *Trebius*, which represent the two extremes in the above series, is particularly instructive. The two-way respiratory current in the gut of *Trebius* is complicated by the muscular action of the relatively powerful mouth parts, the trituration of food in the stomach and its periodic expulsion as faeces by the rectum. In *Lernaeocera* digestion is apparently slow, and during this process the gut is closed at both ends, so that the two-way peristalsis is unimpeded by the exigencies imposed upon it in *Trebius*, and a higher frequency is observed. The freedom from extraneous movements enabled the characteristics of the peristalses of *Lernaeocera* to be studied in detail: whereas in *Trebius* the movements had to be analysed to show the preponderance of forward waves (which enables the food to be retained in the gut for a sufficient time for digestion to be effected), in *Lernaeocera* this emphasis on the forward peristalses is very obvious. In the latter type of parasite this inequality is clearly a relict from the earlier stages when peristalsis had to be controlled with a view to the retention of food in the gut in the face of a continuous current via the anus.

ACKNOWLEDGEMENTS

Our special thanks are due to Dr Stanley Kemp, F.R.S., and to Mr E. Ford for reading this paper and suggesting many improvements. We are also very grateful to Dr N. K. Panikkar for critical discussions on the subject matter.

SUMMARY

A study of the distribution of *Trebius caudatus* from four species of *Raja* trawled from two localities of different depths at Plymouth shows that there is a higher infection rate in the deeper water.

Both at Roscoff (France) and at Plymouth the mean total rate of infection is about 20 %, and the larger fish are more often infected than the smaller. Both sexes are equally parasitized and the copepods tend to collect near orifices on both surfaces of the fish (regions where the mucus secretion is maximum). Immature forms were found at Roscoff in the mucus of the orifices themselves. The collections from both places contained about equal numbers of male and female parasites.

Intestinal respiration, a feature common to free living and parasitic copepods, includes the gaseous exchange through the gut wall, the associated movements stirring the oxygen vector (which is either the external medium taken in by the anus, or the food current when this is the blood of the host), and the circulation of the body fluid as a direct result of these movements. The significance of the gut movements, which include the peculiar two-way peristalsis, is examined in detail in *Trebius caudatus* and *Lernaeocera branchialis*:

the former has a functional anus which admits the external medium and the latter has a closed anus and employs the blood of the host as the oxygen vector.

Peristalses in the three sections of the gut in *Trebius* are not synchronous and wide variations are found in the frequency and strength of the waves and also in the duration of the backward and forward phases of peristalsis. Much of the irregularity is due to the presence of food and faeces in the gut and to ancillary muscular activity.

In *Trebius* the maximum number of total peristalses per minute is in the cephalothoracic section of the gut, but the waves are never transmitted to and fro between this and the genital segment, though they are regularly passed forwards from the abdominal gut. The three sections of the gut are virtually separate in regard to peristalsis, which is controlled by three centres situated towards their distal ends. On analysis an increasing residuum of forward over backward waves is found from the fore to the hind end of the body; this is effective in retaining the food in the gut.

In *Lernaeocera* the cephalothoracic gut is practically absent and the peristalses in the abdomen are fitful and not synchronous with those in the large genital segment, where a two-way peristalsis is clearly seen. The peristalses in this species are unimpeded by the alimentary exigencies imposed upon them in *Trebius*, so that the superiority of the forward waves is obvious: they have greater strength, a higher frequency and a longer persistence than those in the backward direction, i.e. those originating from centre 1. This anterior centre has a depressor effect on the backward waves and is the most sensitive to temperature changes: complete inhibition is brought about by previously subjecting the animal to low temperatures.

Peristalsis in both species is controlled by three homologous autonomic nerve centres which have varying powers of stimulation and inhibition. The significance of the emphasis on the forward peristalses appears to be lost in *Lernaeocera*, for it is clearly a relict of the earlier stages when food had to be retained in the gut in the face of a continuous to and fro current through the anus.

The feeding of *Lernaeocera* takes place at rare intervals and digestion is slow. The intake of blood from the host is dependent on the same mechanism which maintains the dynamic osmotic equilibrium between the contents of the gut and the external medium.

The evolution of the modes of respiration in parasitic copepods is summarized.

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A WIRE-ANGLE INDICATOR FOR USE WHEN TOWING PLANKTON NETS

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(Text-figs. 1–4)

The old type of wire-angle indicator, which consisted of a pendulum swinging across an arc of a circle graduated in degrees, and which had to be applied by hand to the wire, was clumsy to use, especially in bad weather, and when the wire led out over the stern of the ship. It needed someone to attend to it, which often resulted in the wire angle not being checked more than once every five or ten minutes when the crew were busy. This instrument was therefore designed to remain in place throughout the tow, and to give a large dial reading which could be easily seen from a distance. On the research ketch *Culver* there is an engine throttle control mounted close to the wheel, and all the man there has to do is to adjust the engine speed so that the large indicator in the instrument remains on the red line. In practice it was found that the wire angle rarely varied more than about ±2°, except in very bad weather, and so the depth of the nets could be regulated very closely. The instrument is oil damped, so that the pitching of the ship and the vibration of

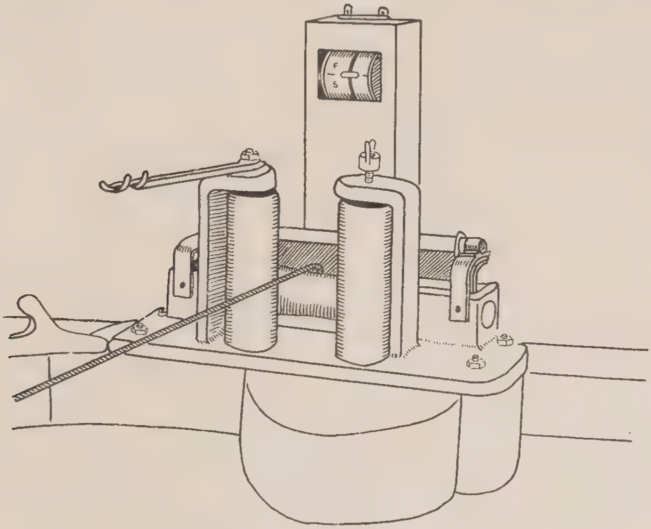

Fig. 1. Angle metre in situ on the stern of the *Culver*.

the wire during hauling do not affect it. It has been used for about two years on the *Culver* at Bermuda; it has needed no attention in that time, and, which is a good point, the crew like using it.

The framework of the instrument consists of two plates ([1]2*c*, 3*c*, 4*c*), spaced apart by three rods (2*i*, 3*h*, 4*e*), bolted on the outside (4*d*). Inside this frame, a pendulum weight (3*f*, 4*f*) is suspended by two arms (3*b*, 4*b*) from an axle (3*a*, 4*m*) which turns in a conical seating in the end of adjustable screws (4*k*) in projections (4*l*) of the main frame. The pendulum is prevented from swinging too far by two stop screws (3*i* and 4*h*) attached by brackets to the main frame. In front of the pendulum is a small block (3*d*), which also turns on an axle (3*e*) in conical seatings in projections (2*j*) of the main frames. This block is coupled to the pendulum by a strip (3*g*, 4*g*), in such a way that a small movement of the pendulum imparts a much larger rotatory movement to the block. The block carries in front a strip (2*m*, 3*l*), on the end of which is the indicator (3*m*) seen on the face of the dial in Fig. 1. On the top of the strip is a flat plate (2*n*, 3*n*), mounted at right angles, and acting as a damper to the swing of the strip.

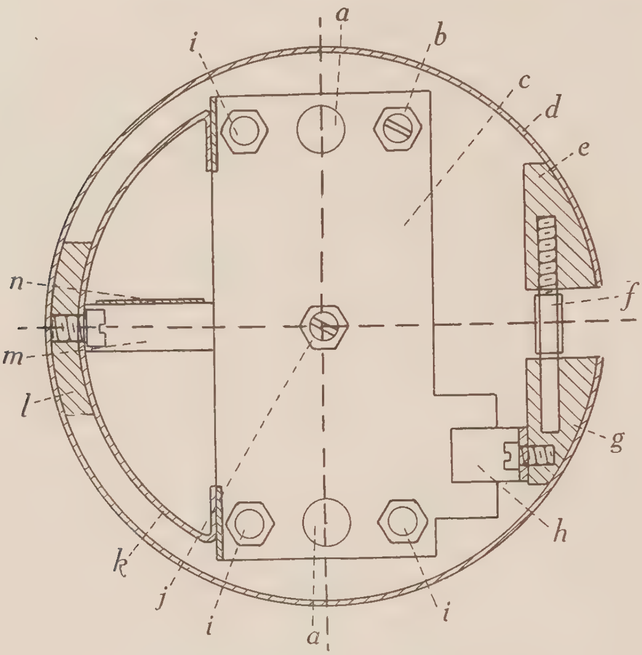

Fig. 2. Section of angle metre from side, taken through outer locking strip (Fig. 4*i*).

The indicator (3*m*) moves over a semicircular dial (2*k*, 3*k*), which is mounted in two halves on brackets on the main frame, and the strip (3*l*) moves up and down in the slit between the two halves of this scale.

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[1] Numbers in brackets refer to text figures.

The whole instrument is mounted in a glass jar, and is held inside this by two locking strips (2*d*, 4*a*, *i*), bent into circles, one at each end of the jar. Each strip is broken at one point, where it has two blocks (2*e*, *g*) attached, and these can be pushed apart by set screws (2*f*, 4*j*), thus tightening the strips against the glass. In assembling the instrument the inner locking strip is inserted first and tightened into place. The framework, attached to the outer locking strip, is then dropped into place, and attached to two brackets (not shown) on the inner locking strip by two screws (3*j*) through the inner frame plate, these screws being accessible through two holes (2*a*) in the outer frame plate. The outer locking strip is then tightened up.

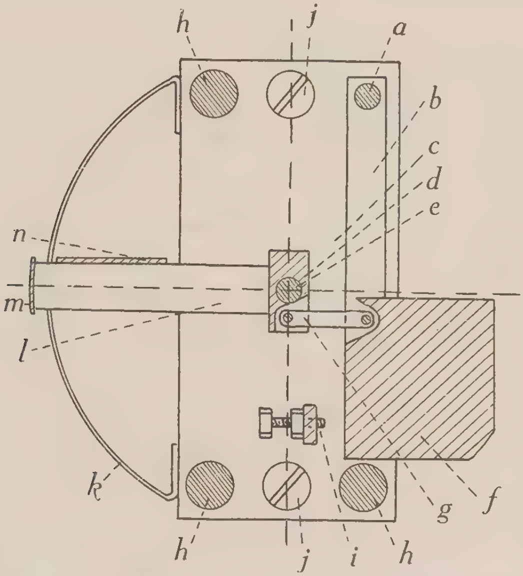

Fig. 3. Section of angle metre from side, and just above centre. The circular locking strips have been removed, as well as the outer frame plate and outer half of dial.

The glass jar is filled with castor oil to damp out too rapid swings, and closed with a glass top and rubber washer, since castor oil does not soften natural rubber. The jar is clamped shut by an outer frame which drops into slots in the wooden outer mounting, and so retains a constant relative angle. As used on the *Culver* the instrument was set to work at a constant towing angle, but the mounting could easily be modified so that this was variable. The bearings of the instrument were made of steel, and the rest, except for the lead pendulum weight, of brass. The dial and indicator were painted with ordinary water colours, which were found to stand up best to the action of the oil, but a baked enamel finish would no doubt be better.

The instrument was mounted in a wooden turret (Fig. 1) on a frame which pivoted horizontally immediately above the roller on which the towing wire passed over the stern of the ship. At the back of the frame carrying the turret was a second roller which rode on the wire, and thus tilted the apparatus with the changing wire angle. The whole apparatus was attached by clamps, and could easily be lifted off when nets were being handled over the rollers, but it did not have to be shifted if wire was being let out or taken in.

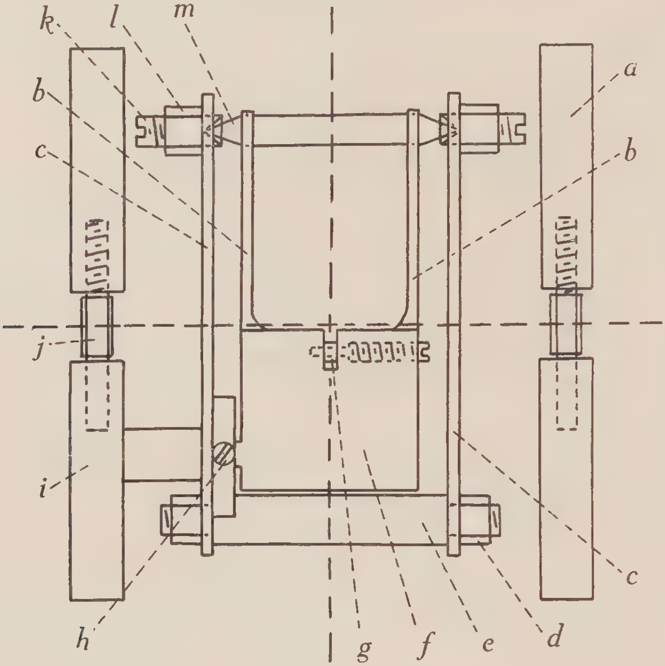

Fig. 4. Rear elevation of angle metre. The parts in front of the pendulum (central block, indicator and dial) are omitted, as well as the brackets attaching the frame to the inner locking strip (*a*).

The instrument as described proved quite satisfactory, and the nets were certainly fished at a more uniform depth after its introduction than with the old hand instrument. It could easily be modified for use with other arrangements of the towing wire, and could be made with the dial at the side or the top if this were required. Actually we had the pendulum weight set far back so that the instrument faced slightly upwards.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1940–41

The Council and the Officers.

During the year the Association has suffered a great loss in the death of Prof. E. W. MacBride who, as Chairman of Council, had presided at the Council meetings since 1928. In the early days of the Association Prof. MacBride was a constant worker at the Plymouth Laboratory and throughout his life he took the greatest interest in its welfare. He had been a Governor since 1924, representing the Zoological Society of London, and in recent years was also Chairman of the Fishery Advisory Committee of the Development Commission.

The Council also regret to note the death of Dr G. Herbert Fowler, who held the post of Secretary to the Association from 1891 to 1895 and for many years gave valuable service as a Council member.

Four ordinary meetings of the Council were held during the year, two of them in the Rooms of the Royal Society, London, two, in October and January, in the Zoological Laboratory, Cambridge; the average attendance at the meetings was twelve. The thanks of the Association are due to the Royal Society and to Prof. J. Gray for allowing the use of the rooms.

The Visiting Committee of the Council inspected the Plymouth Laboratory on 13 April 1940. In June an Emergency Committee was appointed with authority to act for the Council in any urgent matters if it should not be possible to hold the ordinary meetings. At the January meeting, on the motion of the President, Prof. J. Gray was appointed Deputy Chairman of Council.

Air Raid Damage to the Plymouth Laboratory.[1]

Serious damage to the Association's property has been incurred through enemy action during the year under review. In an early raid many windows were destroyed by blast and at a later date heavy damage was sustained by fire and from high-explosive bombs. Two of the latter fell near the front gate, one of them destroying the water main, while another came down on the north side of the Director's house, blew in the wall and brought down a large brick arch, also causing much damage to the south walls of the physiological and chemical laboratories and in the aquarium. At the same time an incendiary came through the roof of the Director's house and though tackled immediately it was not possible to bring the fire under with stirrup pumps. The fire main, which could easily have subdued the outbreak, could not be used owing to lack of water. Help was obtained from the military authorities, who sent a squad of men and a motor pump which could draw from our sea-water reservoirs, and the fire was thus prevented from spreading to the Laboratory as at one time appeared imminent. The Director's house was completely gutted and almost all its contents were destroyed. On the following night three incendiaries struck the premises but were extinguished without causing serious damage: one of these, which was put out with considerable difficulty, fell on the men's lavatories, one in the Common Room and one in the Director's garage.

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[1] See Additional Note, p. 433.

As a result of the attacks practically all the windows are broken except those on the top floor of the south side of the main building, many window frames are demolished or fractured, ceilings have fallen, plaster walls are cracked and bulging and most of the doors have been shattered, split or wrenched from their fastenings. Internal glazing in fanlights and in cupboard doors is for the most part broken and considerable damage has been caused by flying glass. The small store behind the Director's house is demolished, the garage is unroofed and in the Easter Class House the roof is broken and the brickwork at the south-west corner is unsafe. The roof of the dog-fish store is destroyed and that in the constant temperature room has been lifted and the skylights broken. The Aquarium has suffered heavily; a number of the thick plate-glass fronts to the tanks on the northern side have been broken and the supply pipes fractured, most of the larger fish being lost.

The Library is completely undamaged except for the broken skylight and windows, and losses to apparatus and equipment are comparatively light in view of the severity of the attacks. In some rooms furniture has suffered and valuable material in the sales department destroyed.

In clearing the debris, which lay thickly in all parts of the buildings, much assistance was given by visiting workers—Miss N. G. Sproston, Dr M. Parke, Dr N. K. Panikkar, Mr P. G. Corbin and Mr W. J. Rees—and valuable help was also given by a party of students from the Municipal Technical College, organized by Miss F. Stanbury and Mr H. Barnes. The Library was made weatherproof in the first two days and within a fortnight the first and second floors of the main laboratory, with some research rooms in the north building, were habitable. A small amount of glass has been restored, while other windows have been boarded or covered with fibre sheeting from the ceilings of the new laboratories. In the north building old glazed frames and aquarium fronts which had been kept in store have been used to admit light, other parts of the windows being boarded or bricked.

The buildings have been inspected by Mr A. Thorpe, Architect to the Ministry of Agriculture and Fisheries. The most severe structural damage is in the Director's house. With the fall of the large arch on the ground floor of the northern elevation the structure appeared to be in some danger of collapse and the coign to the west of the arch was badly damaged by the explosion. The arch has now been shored, the windows above it strutted, and timbers over the inner side of the windows, which had been burnt through, have been supported. The cut stone south and east elevations of the house are in good condition, though in some places the limestone round the windows will need replacement. The eastern end of the north building is extensively damaged on its southern side; the walls are deeply cracked and heavily scarred by flying debris and the whole building is shaken. The south-east corner has been shored and Mr Thorpe is of the opinion that the southern face at least will have to be rebuilt. The Easter class house will no doubt need rebuilding, and it will not be possible to re-open the Aquarium until after the war has ended.

Mr Thorpe recommends the appointment of a qualified surveyor to prepare the Association's claim for compensation and his advice in this and in other matters is being followed. During the summer a number of repairs either permanent or temporary must be carried out in order to protect the building: slating on the sides of the top floor of the main laboratory must be renewed, new guttering and rain-water pipes must be fitted, asphalt roofs will need attention in order to prevent leaks, and some at least of the outbuildings must be re-roofed.

The water supply was restored about a week after the raid, and electric supply on most circuits failed for the same period; it was, however, possible to keep the sea-water reservoirs pumped up, thus providing a supply of water for any further emergencies. An old $3\frac{1}{2}$ h.p. paraffin engine from the motor boat has now been refitted and installed in the engine room. It can be used to drive the salt-water circulation pumps, serving a small fire hose of sufficient length to reach the upper floors of the library and laboratories.

The Ship and Motor Boat.

As noted in last year's Report the *Salpa* was requisitioned by the Admiralty in December 1939. The rate of hire for the ship is still the subject of correspondence with the Ministry of Shipping, but certain interim payments have been made. All sums received on account of hire will be credited to the *Salpa* Depreciation Fund. The motor-boat *Gammarus* is permitted to work over a restricted area in the Sound and Cawsand Bay.

The Staff.

Five members of the scientific staff are away on National Service. Mr F. S. Russell has a commission as Flight Lieutenant in the Intelligence Branch of the Royal Air Force, Dr L. H. N. Cooper has an appointment in the Ministry of Supply, Dr A. Sand and Mr G. A. Steven have commissions in the Royal Naval Volunteer Reserve and Mr G. M. Spooner has an appointment at the Foreign Office. Mr E. Ford and Dr W. R. G. Atkins hold commissions in the Home Guard and Mr D. P. Wilson is giving voluntary service in the photographic department at the Police Headquarters in Plymouth.

Of the clerical and technical staff four are on National Service: J. H. Bowden (R.A.F. photographic branch), A. Stoate (balloon barrage), A. Mattacola (Royal Tank Corps) and W. H. Gladwell (Royal Marines Police). Of the others three are in the Home Guard, one in the Auxiliary Fire Service, one in the St John's Ambulance Brigade and one is an Air Raid Warden. The engineer-caretaker, E. S. Jope, has reverted to service as a naval officer.

Mr A. J. Smith.

During the year the Laboratory has suffered a heavy loss in the death on 29 January 1941 of Mr A. J. Smith, who had been a valued servant of the Association for 43 years. As Chief Laboratory Attendant he had for a long period done much to further the progress of research at Plymouth.

Occupation of Tables.

The following have occupied tables at the Plymouth Laboratory during the year:

H. Barnes, Plymouth (Hydroxylamine and nitrate degeneration in sea water).
Dr A. M. Bidder, Cambridge (Library).
Dr G. P. Bidder, Cambridge (Library).
W. Thorpe Catton, Chelsea Polytechnic (Haematopoiesis in Teleosts).
Mrs P. G. Corbin, Plymouth (Library).
P. G. Corbin, Plymouth (Mackerel research).
L. R. Crawshay, Plymouth (Sponges).
Miss V. J. Foote, Nigeria (Shore ecology).
Dr V. Fretter, London (Formation of the egg capsule in Stenoglossa).
A. T. Goodman, Exeter (Helminth parasites of fishes).
A. Graham, London (Alimentary canal of stenoglossa).
R. J. Harrison, Kent (Life-history of caprellids).
Dr T. J. Hart, Discovery Committee (Phytoplankton).
P. H. T. Hartley, Westmorland (Library).
Dr M. W. Jepps, Glasgow (*Polystomella*).
R. Jones, Anglesea (Museum).
F. Knowles, Marlborough (Colour changes in crustaceans).
A. G. Lowndes, Rugby College (Density of aquatic organisms).
Miss M. Mare, Cambridge (Micro-fauna and -flora of mud deposits: phytoplankton).
Dr H. B. Moore, Bermuda (Plankton).
Dr N. K. Panikkar, London (Osmo-regulation in marine animals).
R. Pike, Reading (Nervous system of *Galathea*).
W. J. Rees, Millport (Hydroids and hydromedusae).
P. B. Richards, Truro (Oyster culture).
The Hon. Miriam Rothschild, London (Trematode parasites in *Hydrobia*).
Miss H. G. Q. Rowett, Plymouth (Antifouling research).
Dr J. E. Smith, Cambridge (Nervous system of asteroids).
Miss N. G. Sproston, London (Metazoan parasites of fishes).
Miss F. A. Stanbury, Plymouth (Silica uptake by diatoms).

The usual Easter Vacation Courses in Marine Biology were conducted by Mr D. P. Wilson and Mr G. A. Steven. They were attended by thirty-nine students from the Universities of Oxford, Cambridge, London, St Andrews, Aberdeen, Edinburgh, Wales (Bangor), Birmingham, Sheffield, Bristol, Southampton and Hull.

During the Easter Vacation Mr I. T. Hamilton brought one student from Dauntsey School, Mr J. M. Branfoot three from Oundle School, Mr W. L. Francis two from Repton School, Mr A. A. M. Gardiner two from Radley College, Mr A. H. Lewis six from Wellington College and Mr G. C. M. Harris five from Monkton Combe School. The Autumn class was abandoned owing to the prevalence of air raids in the Plymouth area.

The Scientific Work of the Plymouth Laboratory Staff.

It is unfortunately necessary to record a diminution in the research activities of the staff as a result of the war. In addition to the loss occasioned by the absence of a considerable part of the staff on National Service, conditions do not favour concentration on problems in marine biology, and the auxiliary services have made demands which have inevitably interfered with work in the Laboratory. At one time it was hoped that the staff might be able to work together as a team on problems of some national importance, but efforts to bring about such an arrangement have not hitherto been successful. In a number of matters, however, the members of the staff have been able to make small contributions to the national cause, the most important, perhaps, being the assistance which Dr Atkins has been able to give to the Admiralty as well as to two Committees concerned with questions of corrosion and fouling. In addition Dr Atkins, assisted by Mr Russell and afterwards by Mr Steven, has undertaken special work for the naval authorities in the dockyard at Devonport. Under-water tests on the resistance of submarine cables to boring organisms have been carried out and some help has been given on a proposal to utilize seaweeds for purposes of national importance. Information on sea temperatures and other matters has been supplied to service departments. Work has also been carried out by Mr Crawshay and members of the staff on a growth of freshwater sponges which was obstructing the flow in water-supply mains; methods of checking or suppressing the growth have been recommended and experiments are in progress.

During the early part of the year Dr W. R. G. Atkins worked upon a meteorological instrument for the Royal Air Force and prepared for publication the tests carried out upon the preservation of ropes, trawl twines and nets since 1936. Later on he was consulted by the Admiralty Corrosion and Fouling Committee and by that of the Iron and Steel Institute. The action of sea water and of fouling organisms upon various materials was studied. Advice was also given to the Water Pollution Board's staff and an instrument required for a special investigation was re-tested and issued on loan. The Admiralty work on fouling and corrosion is still in progress. A few estimations of the phosphate content of sea water during the winter maximum period were also made.

In view of the direct relationship, found by Russell, between the abundance of young fish and plankton off Plymouth and the quantity of phosphate occurring in the water when at its maximum during the winter, experiments have been made by Dr H. W. Harvey on the quantity of phosphate set free in sea water during storage, and also when the organic phosphorus compounds in solution in it are hydrolysed to phosphate by heating with acid. The aim is to evolve a method of assessing the quantity of phosphorus in a sample of water, collected in summer when the phosphate content is low, which would in nature be converted into phosphate in course of time. It is thought that this quantity may provide an index of the 'potential fertility' of the water, and that its magnitude may differentiate one body of water from another. It is known that when sea water is stored in bottles there is a slow increase in phosphate content, often preceded by an initial decrease; the latter is due to utilization of phosphate by bacteria which multiply rapidly during the first few days of storage. Experiment has shown that phosphorus utilized by bacteria is almost entirely returned to the water as phosphate within three weeks. It appears that the quantity of phosphate liberated in stored water is less, or often less, than would be liberated in nature and that the liberation is due in part to the action of phosphatase enzymes since it takes place in water saturated with chloroform. The quantity of organic phosphorus compounds in solution which are hydrolysed to phosphate on treatment with acid have given interesting but anomalous results. Some evidence has been obtained suggesting that the rapid multiplication of bacteria, which takes place when sea water is kept in a glass vessel, is due to adsorption of organic matter on the glass surface, particularly at the meniscus, where it provides a localized concentration of food for their growth.

Last year it was noted that Mr D. P. Wilson had established clone cultures of the two main forms of *Nitzschia closterium*, the normal spindle-shaped cells and the tri-radiate cells. Cultures of both types remained pure—apart from producing the spineless forms mentioned below—through several stages of sub-culturing and for several months. Eventually a very small number of tri-radiate cells appeared in later sub-cultures of normal ones, and spindle-shaped cells in tri-radiate cultures. Investigation has thrown some light on the manner in which change in shape takes place, although the cause is not known. In cultures of *Nitzschia*, especially those approaching the end of their growth, spineless cells, more or less oval in shape, are produced by division from the spined forms. These spineless cells themselves divide and thereby increase their abundance. If a few such cells be isolated into fresh culture media they usually continue to divide for a time, sometimes giving rise to large numbers of spineless cells. In course of time spined forms are produced by a process of gradual enlargement, a phenomenon at present imperfectly understood. A striking feature is that spineless forms which have originated from tri-radiate cells give rise mainly to normal spindle-shaped forms, although there may be a small proportion of new tri-radiates. On the other hand spineless cells produced originally by spindle-shaped cells have up to the present only been known to give rise to new spindle-shaped cells. The investigation is being continued.

Work on Polychaete larvae, particularly those of the genus *Magelona*, has made some headway but in the main has been seriously hampered by lack of material due to wartime restrictions on collecting. It has been impossible to obtain adults of the especially interesting species *M. cincta*, whose relatively large larvae are the most suitable for sectioning. A few late larvae have been obtained from tow-nettings in the Sound and it is now possible to distinguish in the plankton the larvae of all three species.

Some preliminary experiments with the larvae of a species of *Teredo* have shown that they are generally unable to metamorphose unless allowed to settle on wood, or perhaps some similar substance such as paper. The capacity of the larvae to metamorphose endures for a period of about a week; if at any time during that period they come into contact with suitable timber they are able to settle and subsequently bore into the wood. If the larvae are kept continually in clean glass vessels it appears that ability to metamorphose is eventually lost and they soon die. These experiments extend to the Mollusca principles already shown to hold good for some annelids. They are being continued whenever it is possible to obtain larvae.

During the summer of 1940 Mr Wilson assisted in a technical capacity with the rearing of oysters on a commercial scale in the tanks of Mr J. Kingcome on the River Yealm. An excellent spatfall was obtained and many thousands of young oysters are growing well. They are likely to be of real value to the fishery at a time when it is cut off from its usual sources of supply in Brittany.

Miss Lebour has finished the main part of the work which she did in Bermuda and has published part of it, the remainder being in the hands of the publishers. Two papers have appeared in the *Journal* of the Association: on the larvae of the Pandalidae and the larvae of the British species of *Spirontocaris* and their relation to *Thor* (Crustacea Decapoda). Both of these combine work done at Plymouth with Bermuda work. With Dr R. Gurney a report on Sergestid larvae has been published in the *Discovery Reports* and a further paper with him is in proof in the *Journal of the Linnean Society*. This includes an account of the larvae of Hoplophoridae, Stenopidae, Palaemonidae and others. A paper on larval crabs hatched in Bermuda and another on larval molluscs, especially those from the open waters of Bermuda, with notes on the eggs of the Bermudan Mollusca have been accepted for publication in *Zoologica*, and a paper on larval *Processa* from Bermuda, which affords interesting comparisons with the Plymouth species, and another on larval Axiids, are to be published in the *Annals and Magazine of Natural History*.

Miss Lebour has been working, whenever possible, on the larval Crustacea and Mollusca of Plymouth, continuing her previous work. She has hatched out the little-known zoeae of *Portumnus latipes* and partially reared them, and has made notes on the early crab stages of *Portumnus* and a number of rare Brachyuran larvae. The larvae of *Porcellana* are also being studied and the life-histories of the two common shore species, *P. longicornis* and *P. platycheles*, have been differentiated and worked out. She has also observed the breeding seasons of the two common species of *Teredo* from the raft in the Sound and found that they are both capable of breeding throughout the year. The larvae and young metamorphosed forms of the small species related to *Teredo navalis* have been investigated.

Miss Lebour has also examined tow-nettings from close inshore in the Sound when it was possible to procure them, keeping an eye on the larval Crustacea and Mollusca, noting the breeding seasons and general contents of the hauls.

Up to the time when he joined the Royal Air Force Mr F. S. Russell continued his work on the Monograph of the British Hydromedusae and his routine observations of the off-shore plankton in the Plymouth area. Now that the *Salpa* is no longer available the regular collection of plankton samples has become difficult. For a time arrangements were made with one of the few remaining trawlers working out of Plymouth, and when this failed, through the kind offices of Capt. C. H. Lush, R.N., samples were for a time collected for the Laboratory by one of the mine-sweeping vessels.

Mr G. M. Spooner, while still at the Laboratory, continued the studies referred to in previous reports, relating to estuarine faunas and the special investigation on species of *Gammarus*. Further samples of gammarids have been examined but the general work on the ecology of estuaries has come to a standstill owing to the difficulties of access to these areas in wartime. The work on the systematics and distribution of species of *Marinogammarus* was completed, and a paper brought out in co-operation with Mrs E. W. Sexton. In this paper all the species have been described and figured in detail, and collections from many localities in the British Isles and Northern Europe have been examined and recorded.

Mrs Sexton is now working on another species, *Gammarus zaddachi*, first described by her in 1912, from freshwater and estuarine material. A remarkable variation was noted in the degree of development of the setose armature, and this appeared to be correlated with the degree of salinity of the water. The freshwater form was distinguished by heavier chitin and dense clusters of long setae, the saline type by thin chitin and few setae. Since 1912 both forms of *G. zaddachi* have been recorded from numerous rivers and their estuaries, and usually confused with *G. locusta* and *G. duebeni*. Owing to its abundance, wide distribution and the variation of form, it is much used in connexion with ecological problems, and for this reason it is important to re-describe and figure the two forms on a larger scale.

Detailed drawings have also been made of *Gammarus locustoides* Brandt, a species formerly considered closely allied to or synonymous with *marinus*, but now recognized as belonging to the genus *Anisogammarus*.

Mr G. M. Spooner has assisted the Air Ministry in measures to reduce the numbers of peregrine falcons, which are a danger to carrier pigeons. Previously existing and newly acquired information on the distribution and habits of the falcons has been supplied, and a team has been formed to deal with the coastal area between Plymouth and Torquay.

Mr G. A. Steven continued his work on the mackerel of the western approaches to the Channel up to the time when he left on National Service. The data for a comprehensive paper embodying the results of three years' observations have now been assembled, but it is doubtful whether it will be possible for him to complete his work while the war continues. It is hoped that the collection of data on the mackerel stocks can be continued during his absence.

A paper by Mr E. Ford on vertebral variation in the herring has been published in the *Journal*. The attributes of the species *Clupea harengus* being plainly visible in every element of the herring backbone throughout its length, it is suggested that corresponding 'hallmarks' of sub-specific forms of herring should be looked for. The discovery of such direct indicators of identity would greatly facilitate the study of local populations of herrings and their migrations. In the meantime it is necessary to persevere in the indirect method of assessing and comparing the extent of vertebral variation among the individuals of statistical samples, using counts along the vertebral series as the bases of comparison. Great caution is needed in the interpretation of the data, since individual variation is very pronounced and bilateral asymmetry of common occurrence, even among backbones having the same total number of vertebrae. An interesting alternative method of counting the keeled scales along the ventral edge of the body from the throat backwards to the anus is given, which is in accordance with the marked degree of meristic agreement between the vertebral elements, the myocommata and fin-radials. In the absence of Mr F. S. Russell, Mr Ford is subediting the *Journal*.

Under arrangements made through the Development Commission Dr Mary Parke, who has hitherto been engaged on algological research at Port Erin, has been working at the Plymouth Laboratory. She has recently been studying the life-histories of certain little-known flagellates which have proved valuable as food for oyster larvae. It is long since any continuous work was undertaken on the algae of the Plymouth area and the new arrangement, by which Dr Parke's services are transferred to the Laboratory for a short term of years, is therefore very welcome to the Council.

The Library.[1]

The thanks of the Association are again due to numerous Foreign Departments, and to Universities and other Institutions at home and abroad for copies of books and current numbers of periodicals presented to the Library, or received in exchange for the *Journal*. Thanks are also due to those authors who have sent copies of their books or papers, which are much appreciated. Accessions to the library, particularly in foreign serial publications, have naturally diminished under war conditions; it appears probable that the normal grant for library purchases, now considerably reduced, will have to be increased when the war is over in order to make up arrears.

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[1] See Additional Note, p. 433.

Published Memoirs.

Vol. XXIV, No. 2 of the *Journal* of the Association was issued in August 1940, and Vol. XXV, No. 1 in February 1941.

The following papers, the outcome of work done at the Laboratory, have been published elsewhere than in the *Journal* of the Association:

Gurney, R. & Lebour, M. V., 1940. Larvae of Decapod Crustacea. Part VI. The genus *Sergestes*. *Discovery Reports*, Vol. XX, pp. 1–68.

Holmes, W., 1940. The colour changes and colour patterns of *Sepia officinalis* L. *Proc. Zool. Soc.*, A, Vol. CX, pp. 17–35.

Holmes, W., Pumphrey, R. J. & Young, J. Z., 1941. The structure and conduction velocity of the medullated nerve fibres of Prawns. *Journ. Exp. Biol.*, Vol. XVIII, pp. 50–4.

Kirtisinghe, P., 1940. The myenteric nerve-plexus in some lower chordates. *Quart. Journ. Micros. Sci.*, Vol. LXXXI, pp. 521–39.

Löwenstein, O. & Sand, A., 1940. The mechanism of the semicircular canal. A study of the responses of single-fibre preparations to angular accelerations and to rotation at constant speed. *Proc. Roy. Soc.* B, Vol. CXXIX, pp. 256–75.

Löwenstein, O. & Sand, A., 1940. The individual and integrated activity of the semicircular canals of the elasmobranch labyrinth. *Journ. Physiol.*, Vol. XCIX, pp. 89–101.

Metten, H., 1940. Studies on the reproduction of the dogfish. *Phil. Trans. Roy. Soc.* B, Vol. CCXXX, pp. 217–38.

Moore, H. B. & Sproston, N. G., 1940. Further observations on the colonization of a new rocky shore at Plymouth. *Journ. Anim. Ecol.*, Vol. IX, pp. 319–27.

Panikkar, N. Kesava, 1940. Osmotic properties of the common prawn. *Nature*, Vol. CXLV, p. 108.

Panikkar, N. Kesava, 1940. Influence of temperature on osmotic behaviour of some Crustacea and its bearing on problems of animal behaviour. *Nature*, Vol. CXLVI, pp. 366–7.

Sand, A., 1940. The mechanism of acustico-lateral sense organs in fishes, with special reference to problems in the physiology of semicircular canals. *Proc. Roy. Soc. Med.*, Vol. XXXIII, pp. 741–50.

Smith, J. E., 1940. The reproductive system and associated organs of the Brittle-star *Ophiothrix fragilis*. *Quart. Journ. Micros. Sci.*, Vol. LXXXII, pp. 267–309.

Steven, G. A. & Corbin, P. G., 1939. Mackerel investigation at Plymouth. *Rapp. Proc. Verb., Cons. Int.*, Vol. CXI, App. 2, pp. 15–18.

Webb, D. A. & Young, J. Z., 1940. Electrolyte content and action potential of the giant nerve fibres of *Loligo*. *Journ. Physiol.*, Vol. XCVIII, pp. 299–313.

Membership of the Association.

Vice-Presidents. During the year the Association has suffered the loss of two of its Vice-Presidents, the Duke of Bedford and Lord St Levan. At the

Annual Meeting in June the names of Prof. J. Stanley Gardiner, Sir Joseph Barcroft and Prof. Walter Garstang were added to the list of Vice-Presidents.

Admiral Sir Aubrey Smith has taken the place of Mr R. Olaf Hambro as Prime Warden of the Fishmongers' Company.

Annual Members. The total number of members of the Association on 31 March 1941 was 313 as compared with 318 in December 1940.

Associate Members. During the year the Association has lost two Associate Members, Dr Alexander Bowman and Mr A. J. Smith. Prof. J. Stanley Gardiner and Sir Joseph Barcroft were elected Associate Members in June 1940.

Finance.

Grant from Development Fund. The Council have again to express their thanks to the Development Commissioners for their continued support of the general work of the Laboratory.

Private Income. The Council gratefully acknowledges the following generous grants for the year:

From the Fishmongers' Company (£600), the Royal Society (£50 and £45 for special apparatus), Magdalen College, Oxford (£25), and the Cornwall Sea Fisheries Committee (£10).

The following sums have also been received as rentals of tables in the Laboratory: The Universities of Cambridge (£105), London (£105), Oxford (£52. 10*s.*), Bristol (£25), Birmingham (£15. 15*s.*), Manchester (£10. 10*s.*), Leeds (£10. 10*s.*), Sheffield (£5); the British Association (£50), the Physiological Society (£30), and the Ray Lankester Fund (£20).

New scales of wages for the technical and clerical staffs and for boats' crews came into force with effect from 1 April 1940, and a war bonus, for which nearly all the members of these staffs were eligible, has also been sanctioned with retrospective effect from the same date. Insurance against war damage under the scheme recently introduced by the Government is being effected and for this purpose a new valuation of the Library is being made.

Building Scheme.

The final settlement of the accounts for the Laboratory extension and improvement scheme shows that a deficit of £54. 3*s.* 2*d.* has been incurred. This amount has been met by transfer from the general funds of the Association.

ADDITIONAL NOTE

Shortly after the end of the year covered by this Report, the Council decided that it would be wise to remove the Library from Plymouth. With the exception of a small working collection all the books have now been transferred to safer quarters. Temporary repairs to the buildings at Plymouth have been carried out and the Laboratory is now able to continue its work. [S. K. September, 1941.]

President, Vice-Presidents, Officers and Council.

The following is the list of those proposed by the Council for election for the year 1941–42.

President

G. P. Bidder, Sc.D.

Vice-Presidents

The Earl of Stradbroke, K.C.M.G., C.B., C.V.O.
The Earl of Iveagh, C.B., C.M.G.
Viscount Astor
Sir Nicholas Waterhouse, K.B.E.
The Lord Moyne, P.C., D.S.O.
Sir Sidney Harmer, K.B.E., Sc.D., F.R.S.
Sir P. Chalmers Mitchell, Kt., C.B.E., D.Sc., F.R.S.
Col. E. T. Peel, D.S.O., M.C.
Lord Mildmay of Flete, P.C.
The Right Hon. Sir Reginald Dorman-Smith, M.P.
Sir Joseph Barcroft, C.B.E., F.R.S.
Prof. J. Stanley Gardiner, F.R.S.
Prof. Walter Garstang, D.Sc.

COUNCIL

To retire in 1942

Prof. D. L. Mackinnon, D.Sc.
H. G. Maurice, C.B.
Lord Rothschild, Ph.D.
J. R. Norman
Morley Neale

To retire in 1943

Prof. D. Keilin, Sc.D., F.R.S.
E. S. Russell, O.B.E., D.Sc.
Prof. E. J. Salisbury, D.Sc., F.R.S.
L. A. Harvey
H. Cary Gilson

To retire in 1944

C. F. A. Pantin, Sc.D., F.R.S.
Prof. T. A. Stephenson, D.Sc.
Prof. W. M. Tattersall, D.Sc.
Prof. C. M. Yonge, D.Sc.
J. Z. Young

Chairman of Council

Prof. James Gray, M.C., Sc.D., F.R.S.

Hon. Treasurer

Guy Wood,* M.B., M.R.C.P., 97 High Street, Hungerford, Berks.

Secretary

Stanley Kemp, Sc.D., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

G. P. Bidder, Sc.D.
The Lord Moyne, P.C., D.S.O.
A. T. A. Dobson, C.B., C.V.O., C.B.E. (Ministry of Agriculture & Fisheries)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Admiral Sir Aubrey C. H. Smith, K.B.E., C.B., M.V.O.
  Guy Wood,* M.B., M.R.C.P.
Prof. E. S. Goodrich, D.Sc., F.R.S. (Oxford University)
Prof. J. Gray, M.C., Sc.D., F.R.S. (Cambridge University)
Sir P. Chalmers Mitchell, Kt., C.B.E., D.Sc., F.R.S. (British Association)
Sir Sidney Harmer, K.B.E., Sc.D., F.R.S. (Royal Society)
E. J. Allen, C.B.E., D.Sc., F.R.S. (*Honorary*)

* Died 4th September 1941.

BALANCE SHEET 1941

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31st MARCH, 1941

	£ s. d.	£ s. d.	£ s. d.
Sundry Creditors			76 13 1
Proportion of Subscriptions Received in Advance		19 18 0	
Grant Received in Advance		150 0 0	169 18 0
Income Tax Suspense Account			53 9 8
Aquarium Guide Printing Fund:			
As at 31st March 1940		21 0 0	
Add: Sale of Aquarium Guides		1 19 0	
		22 19 0	
Less: Expenditure		17 6	22 1 6
Special Apparatus Fund:			
As at 31st March 1940		8 16 11	
Add: Grant received from the Royal Society ...		45 0 0	
Transfer from Constant Temperature Rooms Fund		1 8 0	55 4 11
Mackerel Research Fund:			
As at 31st March 1940		93 17 0	
Add: Sundry Receipts		12 10 0	
		106 7 0	
Less: Expenditure		49 17 4	56 9 8
Buildings Extension Fund:			
As at 31st March 1940		232 4 10	
Less: Expenditure	267 19 6		
Amount transferred to E. T. Browne Bequests Fund, as per Statement annexed ...	18 8 6	286 8 0	
Balance Due to General Fund, as per contra		£54 3 2	
E. T. Browne Bequests Fund, as per Statement annexed			5774 5 8
"Salpa" Depreciation Fund:			
As at 31st March 1940		2655 8 9	
Add: Amount received from Ministry of Shipping on account of Hire		300 0 0	
Interest on Investments, less Tax		47 3 3	
Income Tax recovered		59 19 9	3062 11 9
Composition Fees Fund:			
As at 31st March 1940			157 10 0

	£ s. d.	£ s. d.
Boats and Equipment, at valuation as estimated by the Director at 31st March 1941:		
S/S "Salpa"	2000 0 0	
Motor Boat "Gammarus"	100 0 0	
Nets, Gear and General Equipment	30 0 0	2130 0 0
Laboratory Apparatus, Engines and Pumps:		
At valuation as estimated by the Director at 31st March 1941		4000 0 0
Library:		
As per valuation of Mr Ridgill Trout in January 1941		15750 0 0
Stock of Specimens, Chemicals and Journals, as valued by the Director:		
Specimens	1100 0 0	
Chemicals	150 0 0	
Journals	400 0 0	1650 0 0
Sundry Debtors:		
Sale of Specimens and Journals		267 9 9
Income Tax Recoverable		54 13 4
Prepayments		44 11 2
General Fund Investment at market value as at 31st March 1931:		
£352. 2*s.* 3*d.* Local Loans 3% ... ... (Market value at date £320. 8*s.* 6*d.*)		232 7 10
"Salpa" Depreciation Fund Investments at Cost:		
£590. 6*s.* 0*d.* Local Loans 3%	506 10 9	
£2474. 4*s.* 1*d.* Conversion Loan 3% ... ... (Market value at date £3054. 13*s.* 6*d.*)	2506 1 0	3012 11 9
Repairs and Renovations Fund Investment at Cost:		
£153. 1*s.* 0*d.* Conversion Loan 3% ... ... (Market value at date £155. 14*s.* 7*d.*)		153 18 7
Composition Fees Fund Investments at Cost:		
£18. 8*s.* 6*d.* Local Loans 3%	15 15 0	
£139. 2*s.* 11*d.* Conversion Loan 3% ... ... (Market value at date £158. 6*s.* 11*d.*)	141 15 0	157 10 0
Browne Bequests Investments as per Statement annexed		5774 5 8

	£ s. d.	£ s. d.	£ s. d.
REPAIRS AND RENOVATIONS FUND:			
As at 31st March 1940		205 5 8	
Add: Transfer from Income and Expenditure Account		50 0 0	
Interest on Investment		1 14 11	
Income Tax recovered		1 3 8	
			258 4 3
CONSTANT TEMPERATURE ROOMS FUND:			
As at 31st March 1940		4 1 2	
Less: Expenditure	2 13 2		
Transfer to Special Apparatus Fund	1 8 0		
		4 1 2	
			– – –
M. PARKE FUND:			
Grant received during the year		208 0 0	
Less: Expenditure		318 19 2	
BALANCE DUE TO GENERAL FUND, as per contra		£110 19 2	
CAPITAL RESERVE ACCOUNT:			
Arising from revaluation of Library and other assets			17311 8 2
SURPLUS:			
As at 31st March 1940		7095 19 4	
Less: Deficiency for the year as per Income and Expenditure Account	122 6 6		
Income Tax recovered transferred to Special Funds ...	100 13 0		
		222 19 6	
			6872 19 10
			£33,870 16 6

Note: The above Surplus will be supplemented by receipt of the balance of compensation due from H.M. Government for Hire of S/S "Salpa", the amount of which has not yet been ascertained.

	£ s. d.	£ s. d.
CASH AT BANK AND IN HAND:		
Coutts & Company—Current Account	101 0 10	
Lloyds Bank Limited—Current Account ...	328 16 0	
Cash in Hand	48 9 3	
		478 6 1
RECOVERABLE EXPENDITURE:		
Building Extension Fund as per Contra... ...	54 3 2	
M. Parke Fund as per Contra	110 19 2	
		165 2 4
		£33,870 16 6

E. J. ALLEN
L. A. HARVEY } *Members of Council.*

TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

We report that we have examined the above Balance Sheet with the books of the Association and have obtained all the information and explanations we have required. Capital expenditure on erection of Buildings on Land held on Lease from the War Department is excluded. Subject to this remark we are of opinion that the Balance Sheet is properly drawn up so as to exhibit a true and correct view of the state of the Association's affairs according to the best of our information and the explanations given to us and as shown by the books of the Association.

Shinners Bridge, Totnes, S. Devon.
2nd June, 1941.

PRICE, WATERHOUSE & CO.

E. T. BROWNE BEQUESTS

STATEMENT OF FUNDS AND INVESTMENTS 31ST MARCH, 1941

	£	s.	d.
BUILDING FUND INVESTMENTS at Cost:			
£1069. 9*s.* 7*d.* 3% Conversion Stock	1050	8	5
(Market value at date £1088. 4*s.* 0*d.*)			
LIBRARY FUND INVESTMENT at Cost:			
£1049. 8*s.* 7*d.* 3% Conversion Stock	1030	19	7
(Market value at date £1067. 15*s.* 11*d.*)			
SPECIAL APPARATUS FUND INVESTMENT at Cost:			
£2146. 2*s.* 7*d.* 3% Conversion Stock	2110	4	5
(Market value at date £2183. 14*s.* 9*d.*)			
SCIENTIFIC PUBLICATIONS FUND INVESTMENT at Cost:			
£1609. 11*s.* 11*d.* 3% Conversion Stock	1582	13	3
(Market value at date £1637. 15*s.* 3*d.*)			
	£5774	5	8

				£	s.	d.	£	s.	d.
BUILDING FUND:									
By Balance as at 31st March 1940				996	2	2			
,, Interest on 3% Conversion Stock, *less* Tax	18	5	2						
,, Income Tax recovered ...	17	12	7						
				35	17	9			
,, Transfer from Building Extension Fund ...				18	8	6			
							1050	8	5
LIBRARY FUND:									
By Balance as at 31st March 1940				995	14	10			
,, Interest on 3% Conversion Stock, *less* Tax	17	18	3						
,, Income Tax recovered ...	17	6	6						
				35	4	9			
							1030	19	7
SPECIAL APPARATUS FUND:									
By Balance as at 31st March 1940				2039	14	11			
,, Interest on 3% Conversion Stock, *less* Tax	35	16	7						
,, Income Tax recovered ...	34	12	11						
				70	9	6			
							2110	4	5
SCIENTIFIC PUBLICATIONS FUND:									
By Balance as at 31st March 1940				1529	16	2			
,, Interest on 3% Conversion Stock, *less* Tax	26	17	4						
,, Income Tax recovered ...	25	19	9						
				52	17	1			
							1582	13	3
							£5774	5	8

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1941

	£	s.	d.	£	s.	d.
To SALARIES, including the Association's Contributions to Superannuation				7249	12	0
,, LABORATORY WAGES, including National Insurance and the Association's Contributions to Superannuation				2555	12	4
,, DEPRECIATION OF LIBRARY, including Valuation Fee				368	13	3
,, SCIENTIFIC PUBLICATIONS, *Less* SALES				591	12	4
,, UPKEEP OF LABORATORIES AND TANK ROOMS:						
Buildings and Machinery	208	19	8			
Electricity, Gas, Coal, Oil and Water	353	4	7			
Chemicals and Apparatus	194	16	0			
Rates, Taxes and Insurance	109	3	7			
Travelling Expenses	104	13	8			
Stationery, Postages, Telephone, Carriage and Sundries	194	7	5			
Specimens	131	10	1			
Reinstatement of War Damage	133	11	6			
Air Raid Precautions	12	12	0			
				1442	18	6
,, MAINTENANCE AND HIRE OF BOATS:						
Wages, including Diet Allowance, National Insurance and Casual Labour	976	15	8			
Coal, Water, Oil, Petrol, etc.	5	3	7			
Maintenance and Repairs, with Nets, Gear and Apparatus	87	15	9			
Purchase of Material for Nets for Sale, excluding Labour	13	5	3			
Boat Hire and Collecting Expenses	2	19	6			
Insurance	108	13	8			
				1194	13	5
,, BANK INTEREST (net)				2	19	9
,, TRANSFER TO REPAIRS AND RENOVATIONS FUND				50	0	0
				£13,456	1	7

	£	s.	d.	£	s.	d.
By GRANTS:						
Ministry of Agriculture and Fisheries Grant from Development Fund	10687	2	5			
Fishmongers' Company	600	0	0			
Royal Society	50	0	0			
British Association	50	0	0			
Cornwall Sea Fisheries Committee	10	0	0			
				11397	2	5
,, SUBSCRIPTIONS (excluding Subscriptions received in advance)				432	10	3
,, DONATIONS				56	14	3
,, SALES:						
Specimens	808	19	4			
Fish	–	–	–			
Nets, Gear and Hydrographical Apparatus	49	12	11			
				858	12	3
,, TABLE RENTS (including Universities of Oxford £52. 10*s.* 0*d.*, Cambridge £105, Bristol £25, London £105, Sheffield £5, Birmingham £15. 15*s.* 0*d.*, Manchester £10. 10*s.* 0*d.*, Leeds £10. 10*s.* 0*d.*; Trustees of Ray Lankester Fund £20)				446	0	0
,, TANK ROOM RECEIPTS				126	2	9
,, INTEREST ON INVESTMENTS, *Less* TAX				9	0	0
,, INCOME TAX RECOVERABLE				4	14	4
,, SALE OF M. V. LEBOUR'S BOOK					16	10
,, SALE OF "MARINE FAUNA" OF PLYMOUTH				2	2	0
,, BALANCE BEING DEFICIENCY FOR THE YEAR				122	6	6
				£13,456	1	7

THE DEVELOPMENTAL STAGES OF *LERNAEOCERA BRANCHIALIS* (LINN.)

By Nora G. Sproston
From The Laboratory, Plymouth

(Text-figs. 1–6)

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INTRODUCTION

The remarkable life history of *Lernaeocera branchialis* was first described in detail by Claus (1868*b*), who also recognized the close relationship of the old family Lernaeidae, highly modified though it is, to the Caligidae (1868*a*); more recently this close similarity has been emphasized by Gurney (1934). Though the genus *Lernaeocera* is so common on gadoid fishes, the larval forms from pleuronectids are not frequently recorded; indeed, none has yet been found in the western hemisphere. Andrew Scott (1901) obtained abundant material from flounders from the Piel Hatcheries, but his descriptions are not in very great detail, and he leaves some doubt as to the number of stages passed through during metamorphosis.

Wilson's (1917) account is largely based on that of Scott, from whom he borrows his figures. He gives an original description, however, of a nauplius and a metanauplius of a species which he identifies with *L. branchialis*, which he obtained from a gadoid at Woods Hole. In the opinion of Schuurmans Stekhoven (1936*a*) there are so many points of discrepancy between his description and that of the European forms, that (in 1937) he renamed the

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American form *L. wilsoni* (Wilson, 1917). Stekhoven gives adequate descriptions and figures of many species of the genus (1935, 1936*a*, 1936*b*, 1937), but he concerns himself only with adult (mature) females; though he refers (1935) to the finding of larval forms on *Pleuronectes flesus* off the Belgian coast and again at Helder (1936*b*) he does not describe them.

During the examinations of flounders from inshore waters near Plymouth, referred to in a previous paper (Sproston & Hartley, 1941*a*), about a hundred developmental forms of this parasite were collected, and the study of them has served to elucidate many points which were not clear from Claus's account and at the same time has raised new problems. It has been possible during the present studies to correct some details in the existing descriptions of *Lernaeocera* larvae, and an attempt has been made to follow the exact way in which the peculiar suspensory 'filament' of the chalimus stages arises. A comparison of the length data of the various stages has shown a somewhat unusual type of growth curve.

These investigations have been carried out during the tenure of the University of London Post-Graduate Research Studentship at the Marine Biological Laboratory, Plymouth. My thanks are due to the Director and staff of the Laboratory for their encouragement and practical help, and to Dr R. Gurney, who has read the manuscript and given his helpful advice.

The Nauplius

In a previous paper I have mentioned that it was possible to keep the mature female *Lernaeocera* alive for periods up to 9 days in circulating sea water in the laboratory, provided that no injury was done to the parasite during the dissection from the vascular tissues of the host. When the eggs were ripe and the nauplii began to emerge the parasite was transferred to a plunger jar, and some of the nauplii were taken for immediate examination and their moulting into the copepodid form was observed microscopically. Hatching was also observed, but only one form of nauplius was found and no nauplius was ever seen to moult into a second nauplius stage. The enormously long uniseriate egg-strings hatch very slowly, so that some days may elapse before all the nauplii have been liberated—unlike *Chondracanthus lophii*, whose almost equally long multiseriate egg-strings fragment and hatch within a few hours. Hatching eggs have been found throughout the year, and as concluded elsewhere, breeding is continuous in this as in some other parasitic copepods (Sproston & Hartley, 1941*a*). Unfortunately, owing to war-time fishing conditions, it was not possible to obtain flounders for infection experiments, but the identity of the copepods reared in the laboratory with those found from time to time on the gill-tips of flounders left no doubt that the forms described from the latter habitat were *Lernaeocera branchialis* (Linn., 1767) Blainville, 1822.

It may be mentioned that whereas the nauplii soon after hatching swam freely in the circulating sea water and even tended to prefer the surface levels, when about to moult they sank to the bottom. The resulting copepodids, though actively swimming, also tended to keep near the bottom of the container: they would often cease swimming and crawl, largely by a 'hand-over-hand' movement of their chelate second antennae, which during the more active swimming were kept folded back along the under side of the cephalothorax, and so were invisible from above. This observation may be correlated with the behaviour of the intermediate host, the flounder, and it is interesting in that it supports an analogous observation by Gurney (1930). He found that the nauplii of *Nicothoë astaci* and *Chondracanthus lophii*, after a brief period of activity at all levels in the aquarium, sank to the bottom where they remained until moulting. It may not be insignificant that all three species are about to seek hosts which live on the bottom.

Claus did not describe or figure the nauplius stage of *Lernaeocera*, and though Van Beneden (1861, figs. 7, 8) gave two figures, neither these nor his descriptions are sufficiently detailed to be of much value. Wilson (1917) described two nauplius stages: a more or less rhomboidal nauplius 0·45 mm. long and with a maximum width of 0·40 mm. (pl. 12, fig. 107), and a metanauplius 0·55 mm. long, with a maximum width of 0·25 mm. which occurs near the anterior end (pl. 10, fig. 88). The metanauplius differs in shape from the nauplius, and has two pairs of limb rudiments in the form of bud-like protrusions on either side of a small median process at the hind end of the tapering body. No such form was found in the present material.

Andrew Scott (1901, pl. 4, fig. 2) shows 'a newly hatched nauplius' which is broadly club-shaped and in many ways resembles Wilson's metanauplius, especially in the slight indication of two segments near the narrow truncated hind end; the length is given as 0·45 mm., which agrees with Wilson's rhomboidal nauplius in size.

Fig. 1 represents a typical nauplius from the present material from Plymouth, and though there was rather a wide range of size, 0·345–0·405 mm. (see Fig. 6*A*) —the mean of ten random samples being 0·371 mm. long with a maximum width of 0·2–0·25 mm.—it was markedly smaller than either Scott's or Wilson's material. The general colour is slightly yellowish due to the contained fat globules. Irregular chromatophores occur laterally at about the middle two-thirds of the animal; they are of a dark puce colour, and a still darker pigmentation surrounds the eye. The nerve mass, chiefly below this, is bilobed and stains deeply with haematoxylin. Neither my speci-

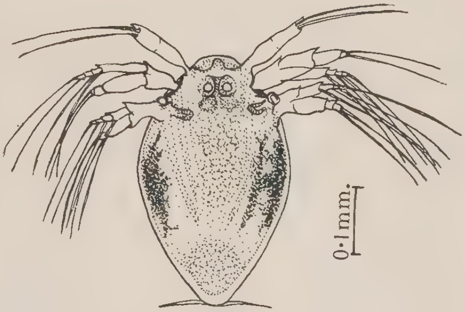

Fig. 1. Nauplius (stage I) of *Lernaeocera branchialis*—dorsal aspect.

mens nor Scott's show limb rudiments near the hind end and I saw no free nauplius showing segmentation, though I have fixed specimens in the process of moulting into copepodids in which such faint segmentation is seen under the skin at the hind end. It seems that in my material the metanauplius stage has been suppressed and is passed through very rapidly just before the emergence of the copepodid. This takes place some 24 hr. after hatching from the egg at laboratory temperatures.

The usual three pairs of appendages are found in the nauplius, though the labrum could not be made out, and a pair of stiff setae, 'balancers', occur at the hind end, usually held at right angles to the body axis, though they have some degree of freedom posteriorly. Pedaschenko (1898, pl. 5, fig. 169) figures them with small articular bosses, but I am unable to confirm this (he also shows small basal joints on the first antennae).

The *first antennae* arise from the sides of a somewhat flattened frontal region by a narrow isthmus, and there is a faint indication of segmentation into two equal joints: they are not so distinct as Scott shows them, and in Wilson's figure of the nauplius the antennae are unjointed and bear two small spines on the posterior subterminal region which I have not seen. In all my specimens there is a short sharp spine on the anterior angle of the tip which also has two long setae. Between these appendages there is a small median papilla under the skin, slightly ventral, which stains darkly with haematoxylin; in nauplii about to moult it is distinctly but minutely papillose. I suspect this of being the precursor of the 'rostral gland' which secretes the peculiar attachment apparatus of the later (chalimus) stages.

The *second antennae* arise somewhat ventrally in a shallow depression on the anterior lateral margins; the basal joints narrow abruptly at their insertion, and there is a short anterior spine on the broad distal end of this joint which gives rise to a four-jointed exopodite and a three-jointed endopodite. The exopodite is more slender and the first joint is about one and a half times longer than the other three together, each of which bears a long seta—four in all. The endopodite is much stouter, and the first two joints are about equal; the first bears a short anterior spine, and the end joint is short and stout with two long setae.

The rami of the second and third appendages have a considerable degree of freedom: Fig. 1 is a camera lucida drawing showing the position of the rami of the second appendage on the right side interchanged; this appendage has a marked tendency to be displaced ventralwards, and the endopodite on the left side is seen in perspective.

The third pair of appendages is similar to the second and the rami bear four and two setae respectively. The proportions of the segments differ slightly, the first joint of the exopodite being shorter and about equal to the remaining three, and the endopodite has a long basal joint and two short ones following it. At the base of the third appendage is what appears to be an excretory gland: a pear-shaped granular mass which stains deeply with haematoxylin and has a non-staining lumen which seems to open on to the basal segment of this limb on its anterior side. In some specimens some indication of a similar aperture could be made out on the base of the second antenna, but no underlying gland was visible.

In his account of the developmental stages of *Thersitina gasterostei*, Gurney (1913, p. 422) states that the maxillary gland was present in the third nauplius (the antennary gland was present in the second nauplius), but even in the later nauplius stages of this copepod he was unable to make out the second maxilla. Owing to the telescoping of the early stages of *Lernaeocera*—a single copepodid following a single nauplius stage—it may be that the antennary gland, which is the normal excretory gland of the copepod nauplius, has only a transitory existence and that it is soon replaced by the maxillary gland of the later stages, this being present even in the nauplius in which there is no second maxilla as yet developed on which it can open. This would mean that at this stage external development is lagging behind internal development. The gland is apparently functional, since it has a clearly defined lumen, and it discharges through a specially formed aperture on the base of the third appendage (the mandible), which in this sense has the functional significance of a second maxilla. Unfortunately, it was not possible to make out the further development of this gland[1] in the copepodid stage, and its aperture has not yet been seen in any of the later stages of this species. When more material becomes available it will be possible to settle this question by serial sections and dissection.

The Free-swimming Copepodid

Large numbers of free-swimming copepodids (Fig. 2*a*) were obtained from nauplii moulting in plunger jars in the laboratory, and others exactly similar were taken from time to time throughout the year on the gill-tips of the flounders caught near the mouths of the estuaries at Plymouth. This stage shows a remarkable variation in size, the length varying from 0·385 to 0·633 mm. with an average of 0·484 mm.; but only three individuals were longer than 0·55 mm., and these will be referred to later when the rate of growth is considered. In spite of the difference in size there was no difference in form of these copepodids, so that whether there is a moult or not in the copepodid stage, it is not thought that more than one stage form can be represented.

The cephalothorax is about five-eighths of the body length and has a strong equatorial line of segmentation behind the second maxillae, and other intermediate folds are present which hint at segmental boundaries. The cephalothorax in all the developmental forms shows a clear ventral infolding along the lateral margins, particularly in the anterior half, as shown in the figures. The mouth-parts in the early stages are not easy to see and they have been omitted from many of the figures. The first antennae are indistinctly five-articled and are abundantly beset with fine setae, the terminal joint bearing four stronger setae; the second joint is the longest, though this is not obvious, especially in the later stages when the first antenna has a tendency to be bent ventrally and laterally along the fold of the cephalothorax, so that in many of the figures it is seen in perspective. These appendages arise immediately in front of the strong chitinized bars which run forward from the anterior lateral angles of the cephalothorax, bifurcate in front, and support the wide basal joints of the chelate second antennae. The second antennae therefore arise only slightly ventrally to the anterior margin of the cephalothorax; they are the main prehensile organs and have a remarkable degree of freedom—rather more than 180°—for at this stage the rectangular rostrum has not yet developed to restrict some slight movement dorsally. Their independent movement in the vertical plane has already been referred to when they crawl over a solid substratum. On finding a gill-tip of a flounder the chelae take a firm hold and this is seldom relinquished, so that it becomes the anchorage of the animal until the adult free-swimming stage (VII) is reached. Owing to the violent currents in the gill chamber the copepod is twisted and twirled on its support; sometimes it is washed off, but though it swims for a time it will soon take a fresh hold. Such currents were imitated for the purpose of observation by removing the gills of flounders to watch-glasses of sea water under the microscope and a one-way current provided by a pipette. The specimen, of which Fig. 2*a* is a camera lucida drawing, was fixed in situ and shows the left chela turned through 180° on its own axis, a compensatory movement just before the animal lost its hold. The gill filaments were grasped by the now well-formed second maxillae, so that the mouth-parts were kept in close contact with the tissues on which they browse, in spite of currents.

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[1] Colonel R. B. S. Sewell, with whom I discussed this matter, made the interesting suggestion that it is more likely to represent the primitive segmental organ of the mandibular somite which has become the functional excretory organ in this type.

Two pairs of well-formed biramous swimming legs are present, the first on the last thoracic segment fused in the cephalothorax and the second on the first free thoracic segment. The second free segment is of characteristic shape and bears two long stout spines on its posterior lateral corners which represent the third pair of legs; the third free segment is also characteristic in shape and represents the pregenital and genital segments; the fourth is the abdomen and bears two relatively large caudal laminae each with five long setae. The exopodite and endopodite of the first pair of legs are two-jointed and the first joint is produced into a fine spine; the distal joint of the exopodite in both limbs bears an outer spine and five long setae, but the endopodite in both bears five long setae only. In the second pair of legs the rami are as yet only one-jointed, but the short spine between the joints of the adult limb is already formed.

THE FIRST CHALIMUS STAGE AND THE SUSPENSORY MECHANISM

At the time of moulting from the copepodid a chitinous secretion appears to come from the mid-frontal region and to be extruded as a laterally flattened thread between the now closely clinging second antennae into the surrounding gill tissue. It penetrates the latter in two diverging filaments—apparently through the perforations made by the claws. The rest of the secretion falls dorsally and laterally forming the hood which is at first attached to the newly formed cephalothorax, enclosing in it the new second antennae and the distal joint of the old pair which remains attached to the gill, inside the hood, and embedded in the chitinous mass. Ventrally the secretion falls as a pear-shaped mass to the level of the chelae of the new antennae.

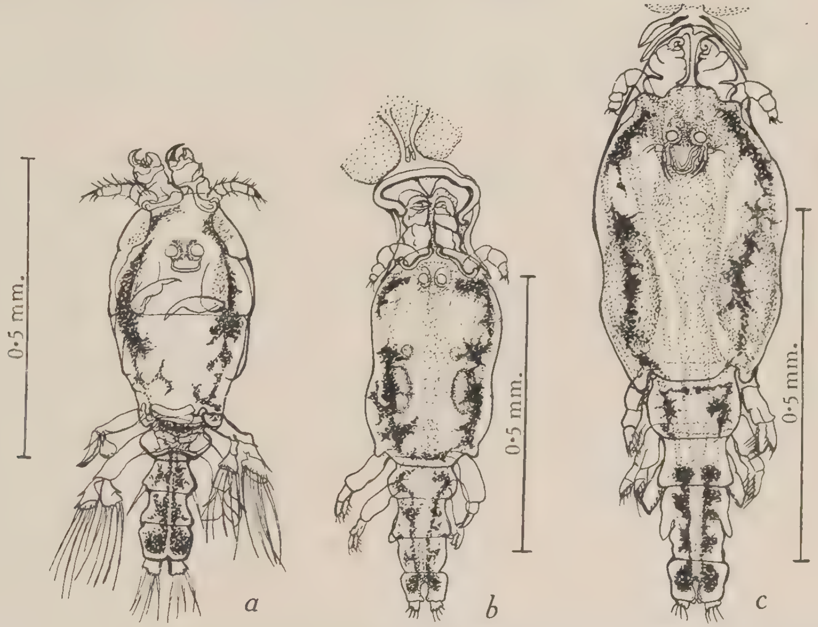

Fig. 2. *a*, Free-swimming copepodid (stage II)—ventral aspect of female. *b*, first chalimus (stage III)—dorsal aspect of male. *c*, second chalimus (stage IV)—dorsal aspect of male.

Soon after this moult has been completed, the movement of the chalimus causes the hood to crack along the transverse line joining it to the dorsal region of the head, and occasionally the dorsal triangular hood section breaks off, but usually the hoods remain attached by their apices to the common filament and the particular stage can be determined by counting these, as Claus has already shown (1868*a*). For example, the fourth chalimus (Fig. 3*b* below) has four hoods, that of the last moult being still attached to the carapace.

Fig. 2*b* shows the first chalimus stage with hood (which was formerly attached to the dorsal region of the copepodid during the moulting period) broken away, but the old chelae are hidden under the chitinous plate: the new hood has broken away except for its lateral edges. The two intrusions of chitin into the gill tissue are seen and in between them is a fine filament—the axial thread of the suspensory apparatus. The ventral pear-shaped masses are best seen in the lateral view of the fourth chalimus stage (Fig. 3*b*).

It seems, therefore, that it is justifiable to refer to the fixed stages of *Lernaeocera* and its allies as 'chalimus stages', since in all essentials they are so similar to the chalimus stages of the Caligidae. Gurney (1934) has given an admirable account of the developmental stages of *Caligus labracis* and *C. centrodonti* and the mode of attachment to their host, but in these as in other Caligidae the arrangement is much simpler, for the sole attachment is by a long filament, secreted once and for all by the first chalimus at the time of moulting from the copepodid. It suspends the animal well away from the host tissues and none of the appendages are involved as they are in *Lernaeocera*; the only addition at each succeeding moult is a small bulb cementing the new frontal region on to the last bulb, and the number of bulbs is an indication of the stage.

A curious intermediate condition is found in *Pennella varians* as illustrated by Wierzejski (1877, pls. 32, 33). These larvae were found on cephalopods and they are very similar, except in the shape of the forepart of the cephalothorax and the wider spread of the free thoracic region and limbs, to the parallel forms of *Lernaeocera*. The strong chelate second antennae begin by suspending the copepodid; but on its moulting they appear to lose hold of the tissues and the secretion alone suspends the first chalimus, leaving a small knob at the top of the conical chitinous funnel, which is much narrower than in *Lernaeocera* and does not include the second antennae but is well anterior and dorsal to them. A bulb of secretion is formed at each moult, but no succession of hoods is left as in *Lernaeocera*. Dedifferentiation of the chelae and other appendages and their loss of segmentation is seen in these chalimus stages as in the present material, but whereas this is at its maximum in the second and third chalimus stages in *Lernaeocera*, it begins earlier in *Pennella*—the first and second stages showing maximum dedifferentiation. Similarly, in *Sarcotretes scopeli*, which is probably a near relative of *Pennella*, Jungersen (1913) has shown four chalimus stages which all appear more retrograde than any stages of the other two genera; the second antennae are more ventrally placed in the copepodid and remain ventral and rather feeble until the free pelagic stage which follows the fourth chalimus. In this species there seems to be no question of the involvement of the anterior appendages in the suspension filament, and the arrangement is somewhere between that of *Pennella* and *Caligus*, differing from the latter in that the filament appears to arise from a broader base as in *Pennella* and so appears as a conical thread; but unlike either no clearly formed bulbs are seen, and certainly no succession of hoods as in *Lernaeocera*. Jungersen admits to using caustic potash (KOH) for the removal of these forms from the fins of *Scopelus glacialis*. This drastic treatment may have dissolved some of the more delicate secretion if this is not pure chitin, for he says: 'In all the present pupal stages I find the structure [of the filament] to be identical.'

To return to the structure of the first chalimus of *Lernaeocera* the most striking change is in the loss of the long-swimming setae on the two biramous legs, the change in shape of the free thoracic segments, and the appearance of a stump in the place of the long spine representing the third leg. The anal laminae are relatively smaller and their setae are also short and poorly developed: only four of them could be made out in this and the following stages. The rami of the limbs have lost their fine structure, though those of the second leg are now two-jointed. No new segment has been added to the thorax.

The Second Chalimus: Stage IV

There has been little change in general shape (Fig. 2*c*) since the last moult, but dedifferentiation has increased. The second antennae are swollen masses with their segmentation shown only by folds, and in the legs it is becoming more indistinct, that of the third leg being barely discernible, though this shows a notched inner margin with vestigial setae. There is an indication of the third free segment dividing into the pregenital and genital segments, and the former shows the rudimentary stump of the fourth pair of legs. In the specimen depicted the dorsal part of the hoods has been broken off, but the axial filament is well shown arising below the bases of the antennae and passing up between them. The mouth tube has not joined up and the mandibles, which are relatively long at this stage, are seen at the sides; in the next stage they are seen within the mouth tube which is in the process of being formed. The second maxillae and maxillipedes have been omitted from these figures to avoid confusion. All the chalimus stages are illustrated by males, though there is only a very slight difference in the shape of the hind body in the female and she is always larger (see Fig. 6 A).

The Third Chalimus: Stage V

The general shape of this stage (Fig. 3*a*) is similar to the last, but some of the appendages are beginning to redifferentiate, though the second antennae appear to be more regressive than in the preceding stage and their segmentation is only indicated by notches; the distal ends of the tips of this appendage belonging to previous moults are seen below each hood—the suspensory apparatus is shown in optical section, the dorsal parts being omitted for clarity. The free segments of the thorax are less clearly marked; but there is an indication of the presence of five, and this is confirmed in the next stage (Fig. 3*b*) in which the genital segment of the adult is clearly shown to be composed of two segments. (This has already been formulated for the Caligidae by Gurney (1934).) Fig. 3*a* shows the emergence of the fourth pair of legs, which are clearly two-jointed at this stage though the small distal blade-like joint has no setae.

The Fourth Chalimus: Stage VI

There is again little change in shape (Fig. 3*b*), but the segmentation of all parts is better defined than in the previous chalimus stage; the limbs, though not functional, are beginning to resume their characteristic form and the setae are slightly stronger, as are also those on the caudal laminae and first antennae. The second antennae have again recognizably chelate ends. The mouth tube is nearly closed and the first maxillae can be seen at its sides, though its bipartite structure is not apparent at this stage. The second maxillae are both shown, the distal joints reflexed on the middle ones; they are still rather swollen and lack the characteristic shape of the adult (and copepodid stage). The maxillipedes are relatively even more swollen and are quite unlike the long slender limbs they become at the next moult; indeed, they resemble the adult second maxillae in general outline. They bear no spines or claws.

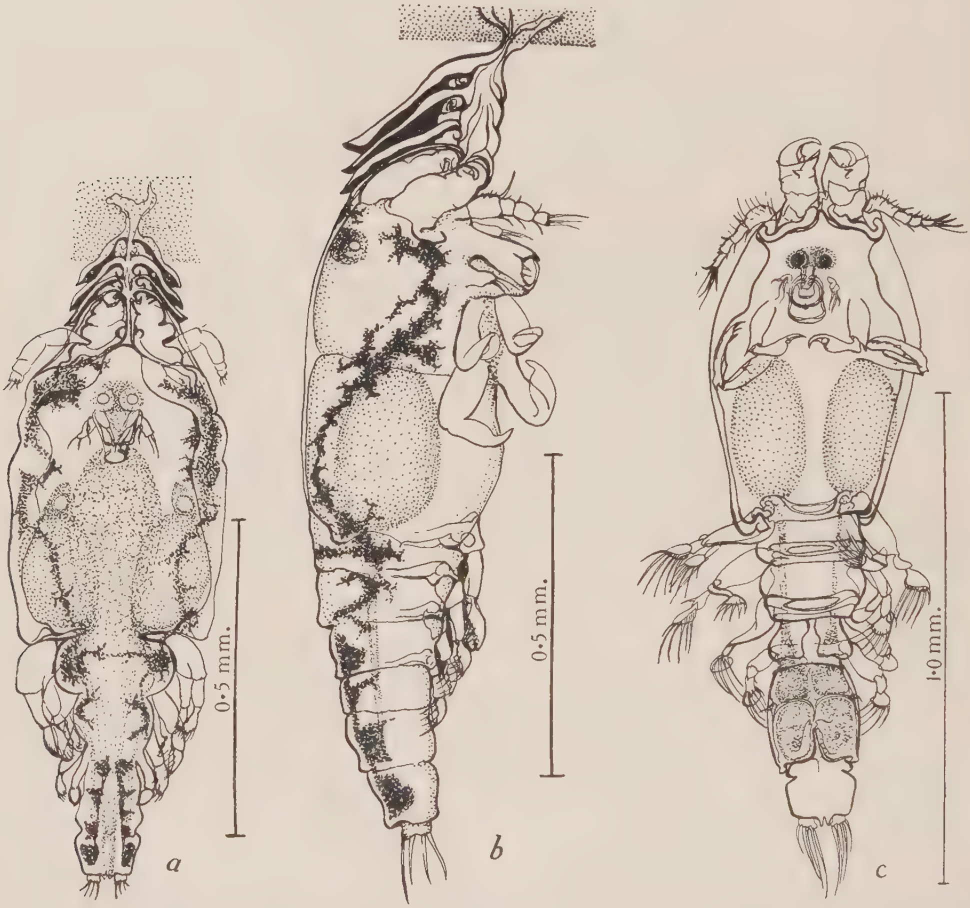

Fig. 3. *a*, Chalimus (stage V)—ventral aspect of male. *b*, fourth chalimus (stage VI)—male from the right side. *c*, adult free-swimming male (stage VII)—ventral aspect, the pigmentation has been omitted.

THE ADULT MALE: STAGE VII

There has been a marked change in shape (Fig. 3*c*) at this moult, and the body form of the copepodid has returned: the setae on the swimming legs are not quite so long, but in fine structure they resemble those of the early stage very closely. The two additional pairs of legs are uniramous and clearly three-jointed; they bear four long setae and a shorter one. Each ramus of the first two pairs of legs bears five long setae and a shorter spine on the outer side, and as foreshadowed in the copepodid each ramus is two-jointed.

The *genital segment* is particularly interesting in the male, for, as mentioned above, its two component segments were indicated quite clearly in the last chalimus stage: they have now coalesced into a barrel-shaped segment containing the spermatophore sacs which open near the median line in two spout-like tubes whose function is not quite clear. It is difficult to make out whether these are the internal openings (? communications with the vas deferens), for they are probably not the external openings for the discharge of the large spermatophores, which Scott holds are beneath the posterior corners of the genital segment. The heavy pigmentation in this region makes it difficult to see the relationships of these structures clearly. The characteristic disposition of the chromatophores has been shown in all the preceding figures, but since the intense dark pigmentation is so heavy in the male, it has been omitted from this figure. That the genital segment does represent two segments is shown in this species almost as well as in the male of *Caligus diaphanus* as figured by Gurney (1934, fig. 17); it is a double segment bearing the vestiges of legs 5 and 6. In the male of *Lernaeocera* the vestige of leg 5 is not easy to see in all specimens, but in some a small spine can be seen arising from a minute boss beneath the cuticle, though sometimes the spine does not reach the surface. Leg 6 is represented by the small indented knob on the ventral side of the posterior corners of the genital segment, but I have not been able to see a seta on this in any of my specimens. Immediately below the genital segment is a short segment preceding the terminal trapezoidal abdominal segment to which it evidently belongs. The anus is terminal and there are two small triangular processes immediately ventral to it; the setae on the anal laminae are four in number, the two innermost are the longest and the two outer, and slightly dorsal, are much finer and shorter.

The first antennae are five-partite as in the copepodid stage and the second joint is longer than the rest, all being richly supplied with short setae; the terminal joint bears four stronger setae and a small curved one. The second antennae are very similar to those in the copepodid, though their relative size is slightly greater and they are very strongly chitinized; they are three-jointed and the terminal joint bears a strong short outwardly directed spine at about the middle of its width on the ventral side.

The mouth-parts. The mouth tube and its immediate appendages do not differ in the two sexes, and they are shown in Fig. 4*a*. It is difficult to state with certainty the exact morphological entities composing the mouth tube, for though it is usually stated that it is formed by the fusion of the upper and lower lips, such an explanation seems too facile. Gurney (1930, fig. 2) figures the mouth tube of the copepodid larva of *Nicothoë astaci* in side view: the lateral suture can be seen, through which the 'masticatory process' of the mandible has already entered the closed tube, while the basal joint of the protopodite remains outside, slightly lateral and anterior to the base of the first maxilla. This lateral suture can be seen in the fourth chalimus stage of *Lernaeocera* (Fig. 3*b*). In Gurney's fig. 4, of the mouth-parts of the adult female *Nicothoë*, a U-shaped chitinous thickening can be seen projecting posteriorly from the dorsal edge of the tube; a similar heavily chitinized rectangular bar is present in *Lernaeocera* (Fig. 4*a*), which seems to be identical with what was identified as the upper lip in the earlier stages (Figs. 2*a*, 2*c* and 3*a*). In the adult it can be traced forwards and inwards, where it bifurcates, the anterior branch passing directly outwards, apparently joining the base of the first maxilla, and the other passing backwards dorsal to the mouth tube and within it forming the lateral borders of the mouth itself, continuing as a flattened concave rim round the lower edge of the mouth aperture to join its counterpart on the other side. The upper border is not similarly fortified, but seems to be flexibly opposable on to the former by virtue of the oblique sheets of muscle fibres in the walls of the mouth, which are attached distally round the orifice. This inner chitinous structure I take to be part, at least, of the lower lip (labium), though it is relatively smaller than this structure in the earlier stages (shown in Fig. 2*c*).

That the labrum and labium are separate entities from the mouth-tube proper, is indicated by Wierzejski (1877) in his figure of these structures side by side in the third chalimus stage of *Pennella varians*. He shows (pl. 33, fig. 16) the tube itself as complete and, apparently separate from it, the labrum and bipartite labium within it. The mandibles are also enclosed in the tube at this stage as they are (for the first time) in *Lernaeocera*. In the mouth tube of the adult *Pennella varians* (pl. 33, fig. 17) the lips are not indicated, nor are they included in any of Brian's figures of the developmental stages of *P. sagitta* (1929). That the mouth tube is more likely to be composed of more than one modified sternite and therefore involves more than the 'paragnaths'—the labrum and labium—is suggested by its enormous development in the mature adult *Lernaeocera* (the figures of which, reproduced in Stekhoven's paper (1936*a*), so nearly agree with my own that they will not be repeated here). Stekhoven's figs. 3*c* and 4 on pl. 2 show the first maxillae carried more than half-way up the oral cone in *L. branchialis*, as do also those of Brian (1929, pl. 5, fig. 9) for *Pennella sagitta*.

If the mouth tube were formed largely from anterior and posterior moieties, its plane of symmetry would be transverse, whereas it is clearly vertical throughout in the adult (Fig. 4*a*). Three pairs of strongly chitinized half-hoops support the structure, which is composed of a thinner membrane with apparent lacunae (or even thinner areas of chitin) along the posterior axis; at least this is so in the adult stages on the flounder (stage VII), for in the mature female, after the final moult on the gadoid host, the mouth tube appears more evenly and far more heavily chitinized. The distal rim of the tube is a narrow membrane edged with minute prickle-like processes. In Wierzejski's figures, referred to above, the three pairs of chitinous bands are seen in an earlier stage of their development in *P. varians* as small projecting lobes within the tube, but not yet applied to its walls. Their late development in this species and their arrangement strengthens my suspicion that part of the mouth tube, at least, represents the highly modified body of the mandibles. 'The masticatory process' of the mandible, which alone is said to be developed in parasitic copepods, is in *Lernaeocera* fairly well developed, but it is very different from that of the Caligidae (and Lernaeopodidae), in which it is a single lancet with an inner dentigerous margin. In the third chalimus stage of *Lernaeocera* (Fig. 3*a*) the mandibles arise from the same origin as the incipient mouth tube, and comprise a stout basal joint with two fine lancet-like setae at its tip. In the adult the basal joint is only seen with difficulty at the bottom of the anterior part of the mouth tube, but the bipartite distal portions are more easily seen as they reach about half-way up the tube. Both rami are slightly concave and about half-way up give off a long sharp spine directed inwards, above which they are sheath-like; the inner ramus ends in a sharp point formed by the rolled-in

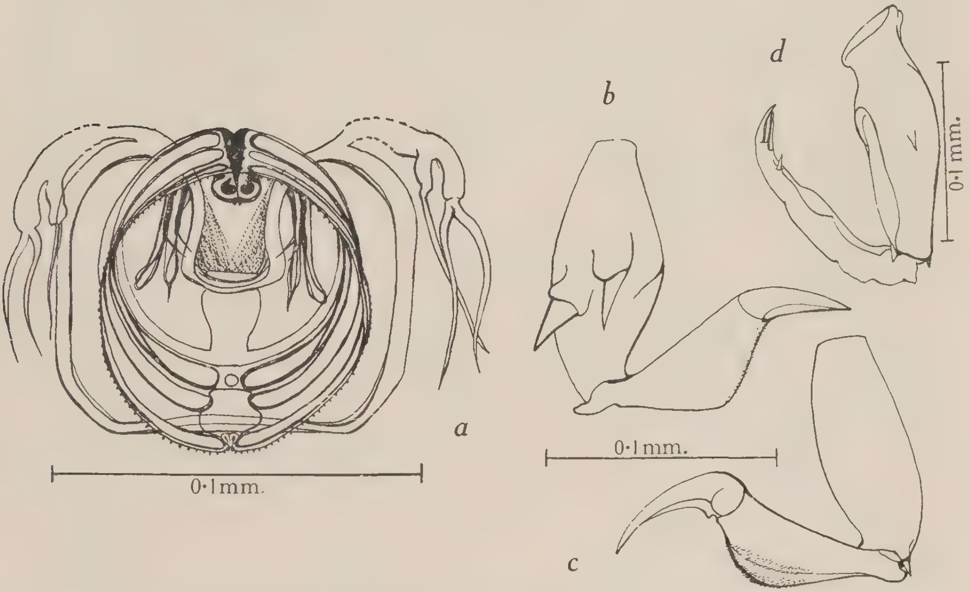

Fig. 4. *a*, Mouth tube in situ, showing first maxillae outside and the bipartite mandibles inside: a composite drawing from stage VII specimens. *b*, right second maxilla of female. *c*, left second maxilla of male. *d*, left maxilliped of male; all from the ventral aspect (stage VII).

edges of the sheath. The outer ramus is an open sheath, and its thickened edges sometimes appear to project as two terminal spines owing to the tip being bent back. I am inclined to think that it was this appearance which led Leigh-Sharpe (1930, p. 336, fig. 5 *Mn*) to depict the mandible of *Lernaea barbicola* (mature female) as a bluntly rounded limb with two short spines: for at certain foci I have obtained almost exactly this view of the outer ramus in *Lernaeocera*. There has been much conflicting description of the mandibles of this group of copepods: Wilson (1917, p. 81), in stating the subfamily characters of Lernaeocerinae (female), says 'the mandibles are unknown', and regarding those of the male, 'antennae and mouth-parts as in the female'. Whereas in dealing with the external morphology of the entire family (p. 24): 'The mandibles are simple curved claws or spines, one-jointed and entirely devoid of teeth'; and in describing *L. branchialis* ('copepodid male') he states 'mandibles in the form of a long and slender spine'. This must have been copied from some previous author, for he does not include males in his material. Even Claus (1868*b*) figures the mandibles, rather indistinctly, as a pair of double broad spines attached to the sides of the mouth tube and within it (which is in no serious disagreement with my own figure).

A. Scott (1901, p. 37) seems to have mistaken the first maxillae for mandibles, for he says they are not enclosed in the mouth tube, though he goes on to say that they are situated at the base of the lateral surfaces of the conical tube of the mouth and consist of two parts, the basal joint being cylindrical and the second joint flattened and terminating in a broad blade, which is serrated on the inner margin. I have seen nothing like this in any of my specimens, nor do Scott's figures support this description. From his figures it appears as one of the broad setae of the first maxilla. Stekhoven neither describes nor figures them; he admits that they are exceedingly difficult to see.

The *first maxilla* (Fig. 4*a*) is in two parts, an inner short joint gradually tapering into a long seta, and an outer joint also tapering off, this time into two long setae. In the chalimus stages the outer joint is relatively larger and stouter (Fig. 3*a*).

The *second maxilla* is clearly three-jointed, and it is particularly interesting in that it is different in the two sexes: that of the female (Fig. 4*b*) bears two very stout spines on the ventral side of the first joint, this also showing a faint transverse fold so that it may in reality be composed of two or three joints. No spines or indication of subdivision into more joints are found in the male appendage (Fig. 4*c*), though a small spine is present on the outer corner of its distal end. The penultimate joint in both sexes is somewhat triangular, and on the outer edge there is a shallow groove, the distal half of which is thickly beset with minute spines—these are rather more extensive in the male than in the female. The end joint is clawed, and in the male it is rather more curved. In the adult (stage VII) of both sexes these last two joints appear to be immovably articulated, and sometimes the suture is difficult to see; this is also true for the copepodid (Fig. 2*a*), but in the intervening chalimus stages the joints lose their characteristic shape and they are flexed on one another (Fig. 3*b*).

The second maxillae, like the first, seem to be remarkably uniform in related genera: the above description agrees almost exactly with the figures of Wierzejski (1877, pls. 32, 33) of *Pennella varians*, where the two spines are shown on the proximal joint of the second maxilla of the female alone. The appendages of Brian's specimens of *P. filosa* (1912, pp. 16–18, pls. 3, 6) and of *P. sagitta* (1929, loc. cit.) are also in agreement in all but minute details.[1]

The *maxillipedes* are only present in the males, and I have not been able to make them out before the fourth chalimus stage; those of the adult male are shown in Fig. 4*d*. They are comparatively long and thin and superficially two-jointed. The proximal joint is the more robust, and like that of the second maxillae may be, in reality, composed of two or more joints, for there is a spine, directed slightly outwards, about the middle of its ventral face; there is also a short spine on its outer angle distally. On the inner face of this joint there is a long concavity and a corresponding one on the inner face of the distal joint which is about the same length. It is thought that this groove has a function in connexion with insemination, and the use of this pair of limbs during copulation is referred to below. There are a number of slight folds along the margin of the distal joint which may indicate its component segments, but they are indistinct. About a quarter of its length from the tip the concavity of the limb ceases, and at this point there is a small spine directed inwards; three longer spines occur at the end—two straight ones subterminally and a curved one terminally, all three being closely opposed.

The Adult Female: Stage VII

Though her own gonads are not yet mature, the female at this stage (Fig. 5*a*) has attained, superficially, the same degree of structural development as the sexually mature male; it is the stage in which copulation takes place, and it is therefore justifiable to call her 'adult'. The ensuing metamorphosis involves superficial dedifferentiation, for the second time, and the final assumption of an entirely different form several times larger than the present one. The cephalothorax is slightly larger than that of the male and is oval, the maximum width being equatorial rather than anterior to the equator as in the male. The appendages are all similar to those of the male with the exceptions already mentioned, and in addition the swimming legs have slightly longer setae and their basal joints are longer and more slender. The distribution of setae differs slightly from that in the male and copepodid stage and is as follows: leg 1, 7 + spine, 6 + spine; leg 2, 6 + spine, 6; leg 3, 6 + spine; leg 4, 5 (in legs 1 and 2 the exopod is cited first).

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[1] It therefore appears that Leigh-Sharpe's figure (1935, p. 108, fig. 1) labelled as the antennule of *Saucissona sauciatonis* is the second maxilla. It shows a three-jointed appendage ending in a blunt claw and having one blunt spine on the basal segment. Apart from the likeness of this figure to a second maxilla of *Lernaeocera* and its allies, its resemblance to the first antenna of any copepod known to me is remote. Leigh-Sharpe (1935) created a new genus for this species (represented by a single immature female) which he considers to be congeneric with *Lernaeocera lumpi* (T. Scott, 1901). Scott's species was described from a single mature female from the gills of *Cyclopterus lumpus*. The separation of the new genus rests on the absence of the familiar flexures in the body of the mature females.

The thoracic segments have lost the characteristic shape seen in the copepodid and adult male, and they are rounded in outline except the pregenital which is slightly quadrangular. The genital segment is without the vestigial appendages of somites VI and VII which were found in the male: it is enormously elongated with a crenulated outline which indicates the region of imminent expansion. Copulation takes place immediately the male comes along, even though the female has barely finished moulting, and she be still

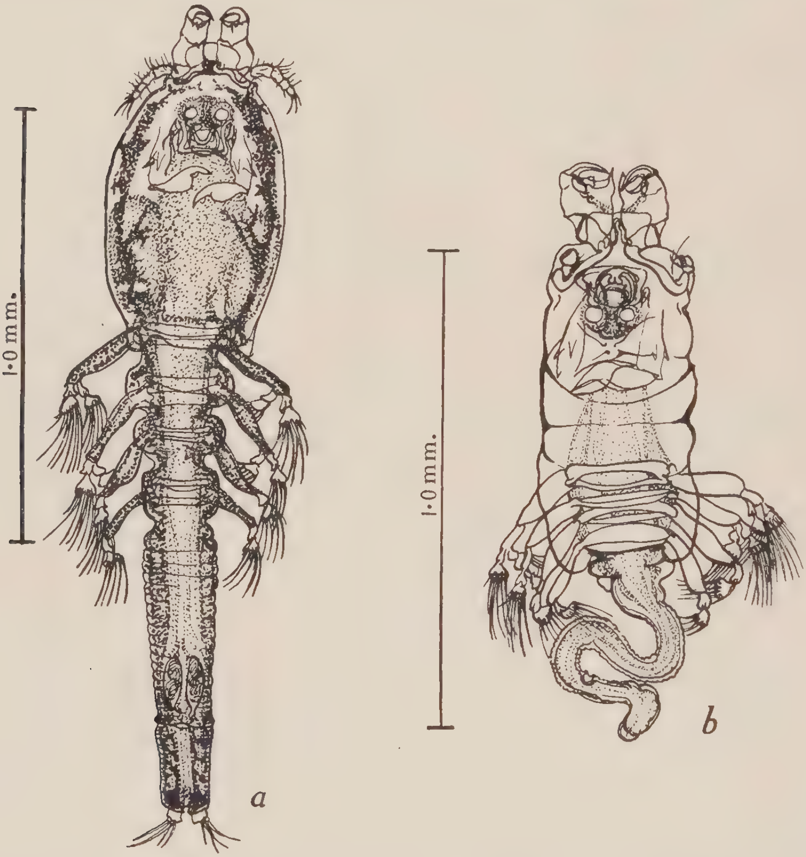

Fig. 5. *a*, Adult female (stage VII)—dorsal aspect, showing spermatophores within the hind body which has not yet expanded to its full length for this stage. *b*, adult metamorphosing female from the gill region of the gadoid host (the first antennae have broken off)—ventral aspect.

attached to the gill in her original position with the chitinous attachment apparatus adhering to the mid-subrostral region. As soon as copulation is over the genital segment elongates very rapidly until it is slightly longer than the rest of the body. By this time the female has let go her hold on the tissues of the flounder and has begun to swim actively; but she does not make much progress, for she is not a good swimmer and relies mainly on currents to waft her out of the gill cavity of the flounder and up to the water inhabited by her next host—a suitable gadoid. From time to time she will use her anteriorly projecting second antennae to rest on the gills of the flounder before quitting them for a pelagic life. The rostrum is rather large and rectangular in the adult and prevents any dorsal movement of the second antennae, which are held rigidly forwards.

During copulation the male first grasps the genital segment of the female, about a third of its length from the proximal end, by means of his chelate second antennae: so firm is his grip on the still soft cuticle of this region that a papilla is raised and some injury done, for when the male retires the papilla persists for a time but finally subsides when the cuticle is stretched in elongation. Specimens fixed at this time and stained show an area of penetration of the dye around the papilla, elsewhere the cuticle is impervious to all but the fiercest stains. Insemination is effected by the male applying himself parallel to the body of the female on her ventral side, which he does with the further aid of the maxillipedes. His body is then flexed vigorously back and forth several times, the spermatophores suddenly appear and are shed opposite to the vulva, where partly by pressure of his body and by the manipulation with the maxillipedes which have now released their hold (the flexed body tearing unmercifully on the cuticle of the female), the spermatophores are introduced into the vulva where they remain intact. I have not been able to see them discharge or migrate higher up the oviducts. The external opening of the vulva is a transverse elliptical fold, but just inside it are two small apertures leading to the oviducts.

The segmentation of the abdomen is not clear; it has several transverse striations and is, like the limbs and thorax, very heavily pigmented. The anlage of the ovaries can be seen indistinctly in the hinder region of the cephalothorax, and the descending oviducts can also be made out. Cement glands appear to be situated in the lateral fields of the genital segment, but details are obscured by pigmentation.

THE ADULT FEMALE: STAGE VIII

I have been unable to find A. Scott's 'pennella stage'—the fertilized female with the long hypertrophied, though straight, genital segment—on the gills of gadoids (Fig. 5*b*). Though Scott found only a single specimen (on the gills of *Gadus merlangus*), Stekhoven has been fortunate in finding what he considers to be a complete series on the gills of the cod (Stekhoven & Punt, 1937). These authors do not figure the specimens showing the formation of the antlers, but they agree with A. Scott (1901) that the latter are produced, and the body elongates (to about eight times the length of the cephalothorax) before flexure begins.

There is, however, some variation in this stage. Stekhoven found one specimen in 1936 of length 13 mm. and a cephalothorax to hind-body ratio of 1 : 8·0; the genital segment had a right-angled flexure and antlers were present. Whereas another found by him on 13 May 1937 had a length of 14 mm. and a ratio of 1 : 8·85, yet the body was straight and there were no antlers. I also found a specimen of total length 14 mm., on 14 November 1940 on *G. pollachius*, in which there was a right-angled flexure in the genital segment, and the second flexure had progressed some 30°; also, not only were antlers present but they had each dichotomized. All specimens found by me on *G. pollachius* and *G. merlangus*, with one exception, have had antlers; and in all the flexure of the body had already begun. There seems to be a moult immediately the impregnated female settles herself on the gadoid, and there may be another later, but the specimen in Fig. 5*b* is, I feel sure, the stage immediately following the pelagic female. I have found only one, on 29 April 1940, situated in the usual position in the anterior angle of the fourth gill-arch of *G. pollachius*. It was hanging on to the tissues surrounding the blood vessels at the base of the gill filaments by means of the second antennae. It was dissected away without damage (except for the loss of the distal segments of the second antennae), and I think it was probably dead, though there were no signs of decomposition. It was fixed immediately, without pressure, in Bouin Duboscq, and no apparent shrinkage occurred. I am inclined to think that moulting had just occurred, as the chitin was very soft and the gut appeared empty; it thus seems that the parasite had probably just arrived on the gadoid after its pelagic (starving) phase and had not yet had its first meal of gadoid blood. It is possible, of course, that this is the final moult and that growth by swelling, on imbibition of gadoid blood, was just about to take place; at all events, the most striking feature of this specimen is the typically flexed genital segment of Stekhoven's later stages, yet the antlers have not yet formed.

Though the cephalothorax is no longer than that of the larger females of stage VII, it is of a different shape, narrower and with parallel sides, and all the leg-bearing segments have become incorporated in it. There are transverse folds across the soft fore-body, and the four radiating folds beneath the nerve mass appear to be the beginning of the dorsal antler, though nothing is visible of the lateral antlers. The first antennae have broken off and their basal joints alone remain. The mouth tube was rather larger than previously and became bent forwards during the mounting of the specimen, and the upper lip is seen above it. The genital segment was very soft and its cuticle more finely crenulated than in stage VII; the opening of the oviduct through which the egg-strings will emerge later is clearly seen at its distal end. It is remarkable to find, at this early stage, that the four right-angled flexures of the mature female are completed. The existence of this specimen is a demonstration of the extreme variability in the order of development of the maturing stages of the female of *Lernaeocera branchialis*. It may be mentioned that the anus appears to be closed in this specimen: the abdomen ends in two lateral flaps, but the lumen of the rectum does not persist to the extremity in mature specimens. The significance of this has been examined at length in a previous paper (Sproston & Hartley, 1941 *b*).

The Rate of Growth

The measurements of over a hundred specimens are represented in graphical form in Fig. 6 A, B. Wherever possible the sexes have been distinguished, but doubtful individuals are shown unsexed. The arithmetic means of the total lengths are indicated for each sex, and the mean of all the specimens at each stage is also shown by a short horizontal line. It is curious that my measurements in all instances should be less than those of Claus (1868 *a*, *b*); but one reason for this is that in expressing the total length of the body I have omitted the projecting second antennae, and where present, the attachment apparatus and rostrum. The anterior limit of the body proper I have taken to be the transverse line across the front of the cephalothorax marked by the bifid ends of the chitinous bars supporting the second antennae. The posterior limit of the cephalothorax is taken at the distal edge of the hindermost coupler of the first pair of swimming legs (except in the specimen in Fig. 5 *b*). The hind end of the body is measured to the tips of the anal laminae, excluding their setae.

In order to test the applicability of Przibram's (1931) law to the growth of the larval stages of *Lernaeocera*, the length of the cephalothorax was chosen as the most convenient part of the body for measurement in all the stages II–VIII; but when it was compared with the total length of the body in all the specimens, the ratios were found to be highly variable. The high degree of scatter of the values for the cephalothorax can be seen in the graph (Fig. 6 B), and the standard deviations of these measurements are given in Table I for comparison with those of the total body length. Owing to the unreliability of the measurements of the cephalothorax, and the fact that it does not show isogonic growth,

Table I. Measurements of the Developmental Stages of *Lernaeocera branchialis*

Stage	Mean length of body mm.		Standard deviation from the mean		Mean length of cephalothorax mm.		Standard deviation from the mean	
I	0·371		0·0145		—		—	
II	0·484		0·0449		0·297		0·0465	
III	0·609		0·0431		0·353		0·0386	
IV	0·732		0·0529		0·426		0·0375	
	♂	♀	♂	♀	♂	♀	♂	♀
V	0·797	0·920	0·0529	0·0183	0·461	0·498	0·1072	0·0302
VI	0·937	1·047	0·0559	0·0277	0·506	0·537	0·0410	0·0683
VII	1·255	1·700 (1·246–1·91)	0·1188	—	0·517	0·619	0·1176	0·0492

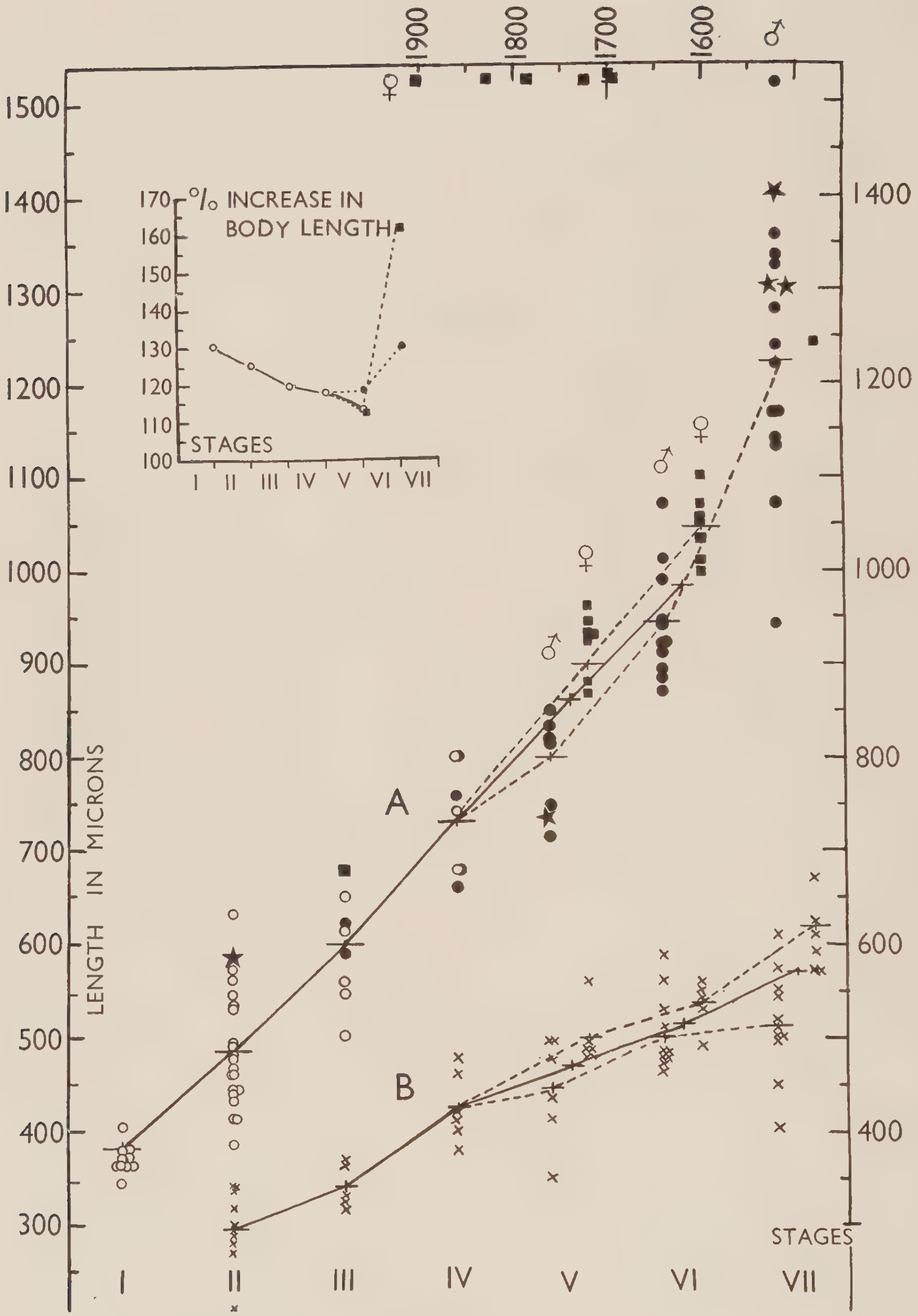
1900
1800
1700
1600
♂
♀
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
1400
1200
1000
800
600
400
170
% INCREASE IN BODY LENGTH
160
150
140
130
120
110
100
STAGES
I
II
III
IV
V
VI
VII
♂
♀
♂
♀
♂
A
B
LENGTH IN MICRONS
STAGES
I
II
III
IV
V
VI
VII

Fig. 6.

the values could not be used in the usual allometric expressions to examine relative growth. Neither could the length of those appendages common to all stages be used, since their measurement in the retrogressive chalimus stages is obviously unreliable.

When the means of the simple lengths of the body, stage by stage, are plotted the result is rather surprising, for instead of obtaining a logarithmic curve—as others have done with the higher Crustacea (Harrison (1940), for example for the caprellid *Pseudoprotella phasma*)—the result is a straight line. The growth approximates to a straight line even when the nauplius is included (stage I) and holds good up to the adult stage VII. There is some irregularity when the sexes are considered separately: the males grow more slowly up to the third chalimus stage, then the curves are parallel until the adult form is reached, and the growth is more rapid. The females grow more quickly than the unsexed earlier stages, and in the final stage on the flounder there is a sudden increase in length of the genital segment, which of course causes the growth curve to bend steeply upwards. In the smallest female shown (1·223 mm. long) the genital segment had only just begun to elongate, and the animal was still attached to the gill tissue by the chitinous apparatus, and though it had only recently moulted fertilization had taken place. However, it is very interesting to note that this specimen is very nearly the same length as the average adult male (see Table I, in which minimum and maximum length are given for stage VII females).

When rate of increase in length is expressed graphically (Fig. 6, inset), it is seen to decrease uniformly up to stages V–VI and then to show a marked increase in both sexes. It is interesting and rather remarkable that this decrease in rate of growth should be so uniform, especially as it is generally assumed that one or more stages are omitted in the early phases of the life history. For instance, there is a marked jump in development, from the structural point of view, between the nauplius and the copepodid with its two pairs of biramous legs and well-developed hind body.

Fresh problems are raised in comparing the rates of growth of presumably related and unrelated copepods. For simplicity, a growth factor has been

Fig. 6. Graphical representation of the growth of the developmental stages of *Lernaeocera*.—A. Shows the total body lengths of the measured series: ordinates represent the lengths in microns and the abscissae the growth stages in seven columns, the sexes being separated into subcolumns V–VII. Unsexed individuals as open circles, males as black circles and females as black squares. The ordinates for stage VII have been continued at right angles along the top of the diagram to include those females in which the hind body had become extended. The arithmetic mean lengths for the total individuals for each stage are shown by a horizontal line and for the separate sexes by a longer line; these values are connected by continuous and dotted lines respectively to show the mean growth-rate. The stars represent the lengths of individuals from hosts other than *Pleuronectes flesus* and are not included in the mean values. B. A similar diagram for the lengths of the cephalothorax: these show a greater variation and a slower growth rate as compared with the body as a whole. Inset: diagram illustrating the rate of increase in body length from stage to stage —see Table II column 1—symbols for sexes as above.

obtained by dividing the length of one stage by the length of the preceding stage and multiplying by 100. In Table II this is shown for *Lernaeocera* (also in Fig. 6, inset), for *Caligus centrodonti* (measurements taken from Gurney, 1934), also for two free-swimming copepods, the fresh-water *Diaptomus castor* (from Gurney, 1940, p. 283, table III) and the marine *Calanus finmarchicus* (from Marshall, 1933, p. 126).

TABLE II. PERCENTAGE INCREASE IN LENGTH OF *LERNAEOCERA BRANCHIALIS* DURING DEVELOPMENT AS COMPARED WITH THAT OF OTHER COPEPODS

Stage	*Lernaeocera branchialis* (Sproston)		*Caligus centrodonti* (Gurney, 1934)		*Diaptomus castor* (Gurney, 1940)		*Calanus finmarchicus* (Marshall, 1933)	
I	131		114		—		—	
II	126		115		134		122	
III	120		154		130		122	
IV	118		157		130		119	
V	♂	♀	♂	♀	♂	♀		
	119	114	178	154	120	131	117	
VI							♂	♀
VII	130	162	—	—	124	119	—	112

As with *Lernaeocera*, the rate of increase in length decreases in the free-living forms *Diaptomus* and *Calanus*, and though the growth factors for the sexed forms vary rather markedly, the gradient of the curve is about the same for *Diaptomus* and *Lernaeocera*, and for *Calanus* it is more uniform though flatter. Gurney (1928) found that the growth factors for *Eurytemora velox* decreased rather rapidly from stage to stage as compared with those of *Diaptomus*. On the other hand, the rate of growth in *Caligus centrodonti*—a parasitic form more nearly related to *Lernaeocera*—increases throughout the life cycle. This increase shows two high jumps: the first is between the first and second chalimus stages when there is no discontinuity in morphological development and only a slight alteration in shape. Between the third and fourth chalimus stages in the female there is a very slight drop in the curve, but for the male there is a further increase in growth rate (but only about half the previous increment) and yet there is again no morphological gap between the two stages; and the change of body form is almost negligible. Such a contrast in the growth behaviour in two nearly related forms is difficult to explain.

It is clear from the foregoing that a discontinuity in the growth-factor curve has no necessary correlation with development: it can neither be used as an indication of abnormal moults (Marshall, 1933), nor of the suppression of certain developmental stages in the life history (inverse induction from the *Lernaeocera* results). Gurney (1928) points out that apart from the growth factor changing from moult to moult, there is a marked individual variation in the free-living copepods which he studied; this has since been confirmed by Marshall (1933) in her work on *Calanus finmarchicus*. Gurney (1940, p. 283, table IV) shows that the growth factor varies irregularly for different parts of the body of individuals of the same sex at the same stage of development, and that these variations are not correlated with the growth factor for the body length, nor this latter with the average of the growth factors for selected parts of the same body.

When the body lengths of *Lernaeocera branchialis* and *Caligus centrodonti* are plotted logarithmically as functions of the growth stages, their difference in behaviour is at once apparent: the curve for *Lernaeocera* is by no means a straight line, but is a curve with a very gentle convexity. That for *Caligus*, on the other hand, is markedly concave—the steep gradient is, however, only developed after the first chalimus stage, and this, incidentally, is the juncture at which accelerated growth is seen in the cephalothorax of *Lernaeocera* (Fig. 6 B).

SIZE VARIATION

From Fig. 6 A the high degree of scatter of the lengths of the various stages is apparent, even though the means of these measurements give a uniform growth curve. It is not possible to group the individuals into two or more size classes in the various stages. At the same time the degree of scatter does not increase uniformly along the stage scale (see column 2, table I) if exception be made for the stage VII females—for which the scatter is due to the operation of a continuous variate within the group—the progressive elongation of the genital segment. The differences in size of the individuals belonging to one stage cannot be explained on the basis of seasonal variation. As an example of this apparently capricious variation in the size of the adult males: an exceptionally small specimen (0·944 mm.) was taken from a flounder on 27 December 1939, while from the same fish another male was taken measuring 1·335 mm. The largest male (1·52 mm.) was found during April, but in the same month other small males were found, including one only 1·075 mm. long.

The appearance of abnormally large copepodids from time to time throughout the year on the gills of the flounder may possibly find their explanation in delayed metamorphosis. Nauplii measured at random from different hatchings in the laboratory showed very little variation in size, and the copepodids obtained from them under experimental conditions had a uniformly small size. It is conceivable that the largest copepodid (0·633 mm. long, found on 13 February 1940) was one that had moulted without metamorphosing, owing to a prolonged pelagic existence in search of a suitable host. Some little support is given to this suggestion by Gurney & Lebour (1941), who found giant larvae of littoral decapods in oceanic plankton, and hint that metamorphosis is delayed owing to the unsuitable habitat: the larvae have got lost, as it were, and when they moult they do so only to emerge as larger forms of the preceding stage.

Whether such giant copepodids give rise to abnormally large chalimids is unknown, though this may be the explanation of the large forms among the later stages in my material (see Fig. 6 A). Attractive as this hypothesis is, it makes the explanation of the abnormally small forms all the more difficult.

LARVAE RESEMBLING *LERNAEOCERA* FROM OTHER HOSTS

During a visit to Roscoff in September 1938 I found a copepodid and a third chalimus stage which in every way resembled *Lernaeocera branchialis*, except that their size was rather wide of the average for these stages (the specimens are marked by stars on Fig. 6 A). I hesitate to identify them with this species because they were found on the gills of *Solea solea* (L.). Since then (Sproston & Hartley, 1941 *a*), numbers of pleuronectids (other than *Pleuronectes flesus* and including *Solea solea*) have been examined carefully for larvae in those localities where *Lernacocera branchialis* is common, but none was found except on *Pleuronectes flesus*.

During April 1940 occasional specimens of *Cyclopterus lumpus* were brought into the laboratory, but the only copepods found on the gills of these fish were three adult males (on one fish) which resembled those of *Lernaeocera branchialis*. The only differences were very slight—in the shape of the cephalothorax—but the specimens were larger than the average for the males of *L. branchialis*. These specimens are also marked by stars on Fig. 6. No character could be found which would separate these larvae into distinct genera or even species, though the specimen referred to above (footnote, p. 455) as found by T. Scott, and since called *Saucissona lumpi* by Leigh-Sharpe, may possibly be the corresponding mature female to these males. On the other hand, it is unusual to find males on the final host, and in the present instance no females were present. The possibility that these males are conspecific with Scott's female cannot be ruled out, but if this is true the almost exact correspondence of the males with *Lernaeocera* shows that Scott's specimen can scarcely be relegated to a separate genus.

At least two examples exist in the Lernaeoceridae of the developmental forms and males occurring on the same host as the mature females: Brian (1929, p. 14) describes all the forms of *Pennella sagitta* on a single host, *Antennarius histrio*, from the Sargasso Sea. A still more surprising record is that of Stekhoven (1936 *a*, pp. 17–18, fig. 16), who found *Lernaeocera lusci* as a mature female in the gill chamber of *Solea solea*, the same host on which I found larval forms of *Lernaeocera* sp. at Roscoff.

Stekhoven suggests that the characters of the second maxillae may prove to be specific criteria in the genus *Lernaeocera*: an excellent suggestion, because these appendages occur well developed in the copepodid, in the free-swimming male and female, and also sometimes in the mature female (very often, however, they are broken off in this stage). I have, unfortunately, been unable to use these characters, for in all my material the appendages appeared identical—except for sexual differences—and I have not found forms exactly corresponding to Stekhoven's figures.

SUMMARY

The morphology of a complete series of developmental stages of *Lernaeocera branchialis* is described. Seven stages are passed through before reaching the gadoid (final) host.

The structure of the mouth tube and appendages is discussed in detail and compared with those of related genera in which the resemblances are close.

The free single nauplius and copepodid stages are immediately followed by four chalimus stages on *Pleuronectes flesus*; these show some dedifferentiation though segmentation is not lost. An explanation is given for the peculiar suspensory mechanism of the chalimus.

A second dedifferentiation follows the second well-developed pelagic phase in which the adult form is attained and copulation takes place. Some irregularity is noted in the details of the retrogressive metamorphosis of the maturing female on the gadoid host.

The rate of increase in body length in *Lernaeocera* decreases regularly up to the assumption of the adult form (stage VII). In this respect it is similar to the free-living copepods *Diaptomus*, *Eurytemora* and *Calanus*, but is in strong contrast to *Caligus centrodonti* which has an increasing growth rate up to the fourth chalimus stage. In the latter at this stage there is a marked acceleration in the growth rate unaccompanied by any change in shape or any other discontinuity, whereas in *Lernaeocera* there are early discontinuities in development, yet the growth rate is continuous when expressed graphically: it is a straight line rather than a logarithmic curve as would be expected from our knowledge of the higher crustacea.

The size of the female is greater than that of the male: that of the female overlapping the male of the succeeding stage. Variations in size of the larvae in a stage group are not seasonal, and an explanation is offered for giant larvae.

Similar lernaeocerid larvae and males to those of *Lernaeocera branchialis* on *Pleuronectes flesus* were found on *Solea solea* and *Cyclopterus lumpus*. No characters of generic or specific significance could be found which would serve to separate them from *Lernaeocera branchialis* so that their identity is not determined.

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ON THE FECUNDITY OF SOME GAMMARIDS

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(Text-figs. 1, 2)

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INTRODUCTION

There is available comparatively little information regarding the fecundity of gammarids. Sexton (1913, 1928) makes some remarks upon the reproductive capacity of *Gammarus chevreuxi*, *G. pulex* and *G. locusta*. Recently she and Spooner (1940) have made an exhaustive taxonomic study of the marine littoral forms, and include brief records of the size of the broods in each species. The present investigation was undertaken with the object of making a statistical study of the fecundity of several species with special reference to the relation between the body weight and fecundity.

The great profusion of marine fauna, particularly of littoral species, on the north coast of Wales is well known (Jackson, 1940). The number of species of *Gammarus* and *Marinogammarus* previously recorded as occurring here is seven, namely, *G. pulex*, *G. locusta*, *G. zaddachi*, *G. duebeni*, *G. chevreuxi*, *M. marinus* and *M. obtusatus*.

In this investigation, the specimens were collected mainly from the upper part of the intertidal zone at the following two places along the Bangor coast of the Menai Straits: (1) the shore on either side of the pier at Bangor, (2) the stream (Gorad-y-Gyt) flowing towards the Straits. Altogether a total of fourteen samples was obtained, nine from the former and five from the latter. The dates of collection are as follows:

Shore collection	Stream collection
Sample I: 27 Nov. 1940	Sample X: 16 Nov. 1940
,, II: 11 Dec. 1940	,, XI: 20 Nov. 1940
,, III: 28 Jan. 1941	,, XII: 22 Nov. 1940
,, IV: 10 Feb. 1941	,, XIII: 9 Dec. 1940
,, V: 6 Mar. 1941	,, XIV: 7 Mar. 1941
,, VI: 19 Mar. 1941	
,, VII: 19 May 1941	
,, VIII: 20 May 1941	
,, IX: 29 May 1941	

An examination of the material reveals the occurrence of six species[1] as shown in Table I.

TABLE I

Species	Habitat	Abundance
M. marinus (Leach)	marine	very abundant
M. obtusatus (Dahl)	,,	abundant
M. finmarchicus (Dahl)	,,	rare
M. stoerensis (Reid)	,,	abundant
G. duebeni Lilljeborg	brackish water	very abundant
G. chevreuxi Sexton	,,	very rare

It will be seen that *M. marinus* and *G. duebeni* dominate the shore and the stream respectively. Not infrequently the former was also encountered in the stream collection. The conclusion that these two species can be found in association has been noted by Beadle & Cragg (1940). It is noteworthy that the distribution of *M. obtusatus* appears to be very restricted in this region, occurring only in a particular area where there is sewage discharge. *M. stoerensis* is usually found in company with *M. obtusatus*, but on one occasion a female was obtained from the stream area. It shows that *M. stoerensis*, though a marine species, prefers to inhabit a place with freshwater influx as already noticed by Sexton & Spooner (1940). All these species are found to be widely distributed in this country, especially *M. marinus* and *G. duebeni* (see Crawford, 1937; Sexton & Spooner, 1940, etc.).

I wish to express my thanks to Prof. Brambell for advice and criticism. My thanks are also due to Mr Cragg for having placed at my disposal a list of species recorded by him and for his kind help in various ways.

BODY WEIGHT AND FECUNDITY DATA

Owing to the extremely small numbers of females which were obtained, *M. finmarchicus* and *G. chevreuxi* are excluded from this investigation. The mean values of the body weight[2] and the fecundity of other species, together with the standard deviations, are shown in Table II.

It will be seen that the mean and the standard deviation of the body weight vary with the species, being highest in *M. marinus* and lowest in *M. stoerensis*. The former is nearly nine times as heavy as the latter.

As regards mean fecundity, the species fall into the following order: *M. stoerensis* > *M. marinus* > *G. duebeni* > *M. obtusatus*, the difference between the first and the last being fifteen eggs per brood.

By comparing these two sets of data, it may be seen that the size of a species bears no relation whatever to the reproductive capacity, as shown plainly in *M. stoerensis*. Although it is the smallest species, its mean fecundity is greater than that of the others. Sexton (1928) obtained the same result from her investigation into the reproduction of various species of *Gammarus*, stating: 'The number of young in a brood varies with the species, but the size of the species is no gauge of either the size or the number of young. *G. chevreuxi* is a small species compared with *G. pulex*, yet it produces up to 63 young in a brood, whilst the highest record for *G. pulex* is only 28.'

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[1] Their identification has been confirmed by Mrs Sexton to whom my thanks are due.

[2] Before weighing, the eggs were taken out of the brood pouch and counted. The animals were then drained thoroughly by means of blotting paper for a certain time depending on the size of the animal.

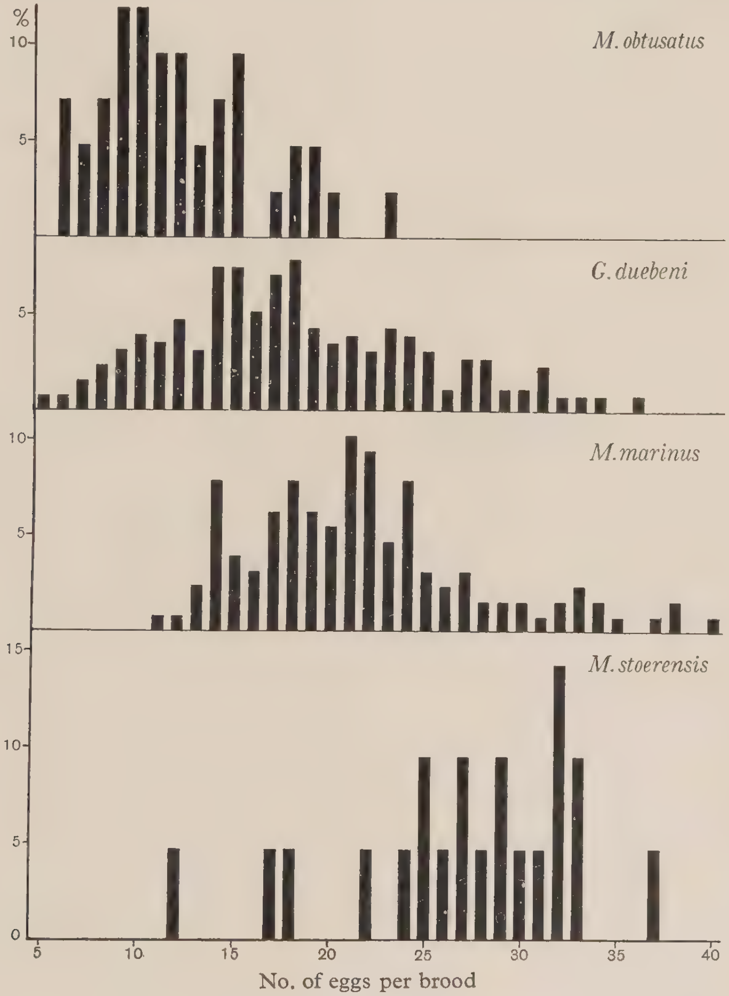

Fig. 1. Percentage frequency distribution of fecundity of various species.

A comparatively small difference exists in the maximum fecundity of the four species examined; the maximum is highest in *M. marinus* with forty eggs per brood and lowest in *M. obtusatus* with only twenty-three eggs per brood.

TABLE II

Species	Number of animals	Body weight (mg.)			Fecundity (no. of eggs per brood)		
		Range	Mean	Standard deviation	Range	Mean	Standard deviation
M. marinus	128	19–63	37·71 ± 0·91*	10·3 ± 0·64*	11–40	21·68 ± 0·53	6·02 ± 0·38
M. obtusatus	42	10–30	18·17 ± 0·64	4·18 ± 0·46	6–23	12·02 ± 0·63	4·09 ± 0·45
M. stoerensis	21	3–6	4·52 ± 0·16	0·75 ± 0·11	12–37	27·1 ± 1·32	6·04 ± 0·93
G. duebeni	254	7–27	15·29 ± 0·21	3·34 ± 0·15	5–36	18·13 ± 0·42	6·63 ± 0·29

* Standard errors.

TABLE III

Species ...	*G. duebeni*			*M. obtusatus*		*M. marinus*		
Sample no.	X–XII	XIII	XIV	I–II	VII–VIII	III–IV	V–VI	VII–IX
Date	16–22 Nov.	9 Dec.	7 Mar.	27 Nov. and 11 Dec.	19–20 May	28 Jan. and 10 Feb.	6–19 Mar.	19–29 May
No. of animals	30	102	122	22	20	15	73	40
Mean fecundity	13·7 ± 1·01	15 ± 0·45	21·8 ± 0·57	10 ± 0·64	14·2 ± 0·9	19·9 ± 1·2	22·3 ± 0·71	20·9 ± 0·97
Standard deviation	5·53 ± 0·71	4·58 ± 0·32	6·36 ± 0·41	2·99 ± 0·45	2·99 ± 0·45	4·64 ± 0·85	6·03 ± 0·5	6·12 ± 0·68

The largest broods recorded by Sexton & Spooner (1940) for *M. marinus* and *M. obtusatus* number fifty and twelve respectively.

The percentage frequency distribution of fecundity in the various species is represented by histograms in Fig. 1. It will be seen that the modal class is by no means the same in the four species, being 9–10 in *M. obtusatus*, 18 in *G. duebeni*, 21 in *M. marinus* and 32 in *M. stoerensis*. This diagram provides a clear picture of the reproductive potentiality of each species.

As already mentioned in the introduction, the collecting period extended from 27 November to 29 May for the 'shore' samples and from 16 November to 7 March for the 'stream' samples. In order to show whether there exists any seasonal variation in the fecundity of various species, another figure (Fig. 2) has been drawn in which each month is represented by a separate histogram. It is evident that the fecundity does vary with season, being generally higher in the spring than in the winter months, as is also shown clearly in Table III. The phenomenon is best displayed in *G. duebeni* in which the modal class for each month is apparently different, being 9 for November, 18 for December, and 23 for March.

That the mean fecundity is on the whole higher in the spring than in the winter is not unexpected, because in the former period external conditions, such as temperature and food, are obviously more congenial. It is found in *Daphnia* that the size of a brood produced by a female in the laboratory can be increased by both higher temperature (up to a certain limit) (Berg, 1931) and rich food (Ingle, 1933). The increase in fecundity in the spring has no connexion with the age of the animal, because an analysis of the material reveals that a female of the same weight generally produces less eggs per brood in the winter. It is unfortunate that the collecting period does not extend beyond May, so that no comparison can be made between the mean fecundity of various months of the year. Such an investigation has been carried out in other animals. Berg (1931), studying the productivity of *Daphnia* in nature throughout the whole year, finds that the maximum fecundity occurs in May.

Practically nothing is known regarding the breeding of these species in nature except in *G. chevreuxi*, which has been found to breed all the year round (Mrs Sexton, private communication). In the present investigation pregnant females of *M. marinus*, *M. obtusatus* and *G. duebeni* were present in all the samples collected, showing that these species are capable of breeding in the winter as well as in the spring.

Relation between body weight and fecundity

The species used in this investigation are *M. marinus*, *M. obtusatus* and *G. duebeni*, because the number of females obtained is relatively larger in these species. Their body weight and fecundity data are shown in Table IV. It will be seen that although there appears to be a great variation in the fecundity of certain body-weight classes, particularly so in *G. duebeni*, the general tendency

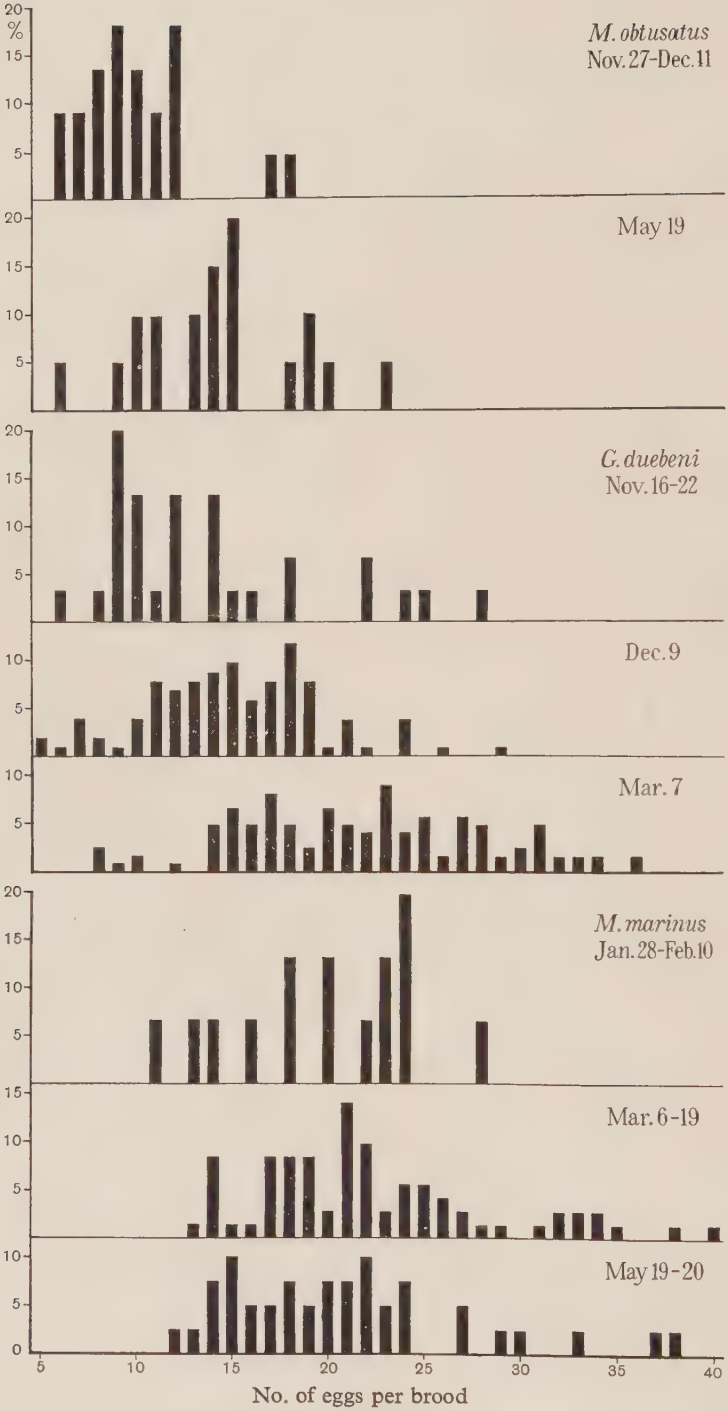

Fig. 2. Monthly percentage frequency distribution of fecundity of various species.

TABLE IV

Fecundity (no. of eggs per brood)	*M. obtusatus*				*M. marinus*										*G. duebeni*				
36–40	.	.	.	.	.	.	.	.	.	1	.	1	2	.	.	.	2	.	.
31–35	.	.	.	.	.	.	.	.	.	1	3	3	1	1	.	2	10	.	.
26–30	.	.	.	.	1	.	1	.	3	3	1	1	2	1	.	5	14	4	.
21–25	.	.	.	1	1	1	4	7	10	10	8	1	2	.	2	22	16	6	1
16–20	.	1	4	1	2	4	13	7	5	5	2	.	.	.	3	41	23	3	1
11–15	3	10	4	.	.	2	9	6	2	1	.	.	.	.	2	42	20	3	.
6–10	9	9	.	.	.	.	.	.	.	.	.	.	.	.	4	18	8	.	.
1–5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.
Body weight (mg.)	10–15	16–20	21–25	26–30	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	6–10	11–15	16–20	21–25	26–30

is towards an increase in fecundity with an increase in body weight. Sexton (1928) obtained the same result from her experimental investigation into the reproduction of *G. chevreuxi*, stating 'the number of young in a brood increases as the female grows'. A comparison of these three tables reveals that the trend is most marked in *M. obtusatus* and least in *G. duebeni*. In the latter the positive correlation between the body weight and the fecundity does not appear to hold for the heavier groups exceeding 20 mg. in body weight. These larger females are found to be present in all the samples collected. No such tendency to reduction in fecundity of older individuals is discernible in the other two species, probably due to lack of sufficient material, especially in *M. obtusatus*.

The relation between fecundity and body weight in the three species is measured statistically by estimating the value of the correlation coefficient. The result is shown in Table V.

TABLE V

Species	No. of animals	Correlation coefficient (r)	Test of significance of r	
			t	p
M. marinus	128	+0·668	7·524	<0·01
M. obtusatus	42	+0·713	4·566	<0·01
G. duebeni	254	+0·383	5·494	<0·01

It is noteworthy that the correlation coefficient of *M. obtusatus* is nearly twice as high as that of *G. duebeni*. It is expected because, in the latter, the positive correlation between the body weight and the fecundity appears to break down after the 19 mg. body-weight class. The observed correlations are definitely significant because p is less than 0·01.

The relation between body weight and fecundity has been studied in other animals by several investigators (Wynne-Edwards, 1929; Gregory, 1932; Stone, 1938). Their result shows that fecundity bears an almost linear relationship to the body weight of the animal studied. The same holds true of the relation between body length and fecundity (Allen, 1895; Stone, 1938; Hickling, 1940). All these investigators agree that the productivity increases as the animal grows. The present investigation reveals, however, that the linear relation between the body weight and the fecundity does not hold for the higher body-weight groups and that fecundity reaches a maximum at a certain body weight or age, as seen in the case of *G. duebeni*. This finding is substantiated by the outcome of the breeding experiments. In *G. chevreuxi*, it is found that after reaching its maximum, the size of broods produced by a female becomes greatly reduced towards the end of the reproductive period (Sexton, 1928). In *Daphnia*, the reproductive capacity drops to a low level after attaining its peak at about the 12th–14th instar (Ingle, Wood & Banta, 1937; Banta, 1939). It thus leads to the conclusion that the correlation between body weight and fecundity is positive at first and then becomes negative after the latter has reached its maximum at a certain age.

Summary

1. The number of species found in this investigation is six, of which four are marine and two brackish water.

2. The mean value of the body weight and the fecundity varies with the species. The size of a species bears no relation whatever to the reproductive capacity.

3. The fecundity is subject to seasonal variation, being generally higher in the spring than in the winter months.

4. In general, a positive correlation exists between body weight and fecundity of the individuals of each species. This rule does not, however, apply to *Gammarus duebeni*, in which the fecundity appears to fall after reaching its maximum at the 19 mg. body-weight class.

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THE AMERICAN WHELK TINGLE, *UROSALPINX CINEREA* (SAY), ON BRITISH OYSTER BEDS

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(Text-figs. 1–10)

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INTRODUCTION

Although it is probable that the American slipper limpet (*Crepidula fornicata* L.) was introduced with consignments of American oysters about 70 years ago (see Robson, 1929; McMillan, 1939), it was not until 1928 that it was known that the American oyster pest *Urosalpinx cinerea* (Say) had also become established on British oyster beds. The first record of the occurrence of *Urosalpinx* is that of Orton (1927) who figured American tingles in an article relating to the native rough tingle *Ocenebra erinacea* (L.), the two forms being confused. Later, Orton & Winckworth (1928) corrected this mistake and definitely recorded *Urosalpinx* for the first time. Subsequently, Orton (1930) has recorded the finding of specimens of *Urosalpinx* among material collected in 1920. It is almost certain that this pest had been established in one or two places for several years before it was recognized. The confusion between *Urosalpinx* and *Ocenebra* still persists among many oystermen, who lump them together as tingles, drills, or borers, different terms being employed in different districts.

In 1939 the writer was informed that several tingles had been found a week previously among American oysters received by an east coast oyster merchant. It is not known whether they were alive, but the possibility of fresh introductions will exist as long as American oysters are imported.

In presenting this paper I have to thank Mr R. E. Savage for criticism and advice and Mr H. H. Goodchild for the photograph on p. 483. I am also indebted to a large number of east coast oystermen who have supplied specimens or information, and in particular to Mr F. E. Wombwell and the

foremen and dredgermen of the Tollesbury and Mersea Native Oyster Fishery Company, who assisted me in every way possible.

DISTRIBUTION

In America *Urosalpinx* occurs from Cape Cod to Florida (Galtsoff, Prytherch & Engle, 1937), while it has also been introduced into San Francisco Bay and to Bermuda.[1] Along the English coast its distribution has not been fully worked out. The two main centres of distribution appear to have been Brightlingsea and West Mersea, Essex (Fig. 1), where for many years American oysters have been laid down. It is very abundant in the River Blackwater and in all the creeks running into the Blackwater to the south of Mersea Island. These creeks are leased to a large number of separate planters, most of whom have at some time or other dealt in American oysters. In Brightlingsea Creeks and in the River Colne *Urosalpinx* is also abundant. Farther south it occurs sparingly in the River Crouch and more abundantly in the River Roach and in the creeks around Paglesham. It seems likely that *Urosalpinx* was recently introduced into the Roach-Crouch river system, probably not long before 1934, for local dredgermen then noted the occurrence of a new type of tingle. These men suggested that they were brought with winkles (*Littorina*) or oysters from West Mersea, the winkles, unobtainable in quantity in the Crouch, being used to keep the oyster pits free of weed. *Urosalpinx* evidently multiplied rapidly, for in one year (1936–7) the company working the grounds in the River Roach paid £9. 5*s*. 0*d*. for tingles collected, at the rate of 1*s*. per 1000, equivalent to approximately 185,000 tingles, many of which, possibly even the majority, would of course be *Ocenebra* and *Nucella*.

On the Kentish coast *Urosalpinx* occurs on the Whitstable beds at the mouth of the River Swale, but it does not thrive on these beds in the same way as in the Essex rivers. The beds at Whitstable are practically in the open sea, and the bottom is hard and quite different from that in the Essex creeks. It clearly does not represent a very favourable habitat for *Urosalpinx*, for it has never become abundant despite the fact that numerous oysters from Essex are laid down annually at Whitstable and small tingles must be carried with them. There is no evidence that *Urosalpinx* occurs in the River Medway, for although I have not dredged there, numerous inquiries among oystermen who work in the Medway, and the showing of specimens, elicited only negative answers, although American oysters have been laid down in the area on several occasions. The beds at Faversham in the River Swale are said to carry no tingles.

Although *Crepidula* now occurs from the Humber to Dorset and the Isle of Wight, and has recently been carried in small numbers to the River Yealm, south Devon, although it may not have established itself there, there are no authenticated records of *Urosalpinx* outside of Essex and Kent. It must be admitted, however, that no intensive search has been conducted in other areas,

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[1] Needler has recently recorded (*Bull.* LX, *Fish. Res. Bd. Canada*) the occurrence of *Urosalpinx* in the Northumberland Strait area of the Atlantic coast of Canada.

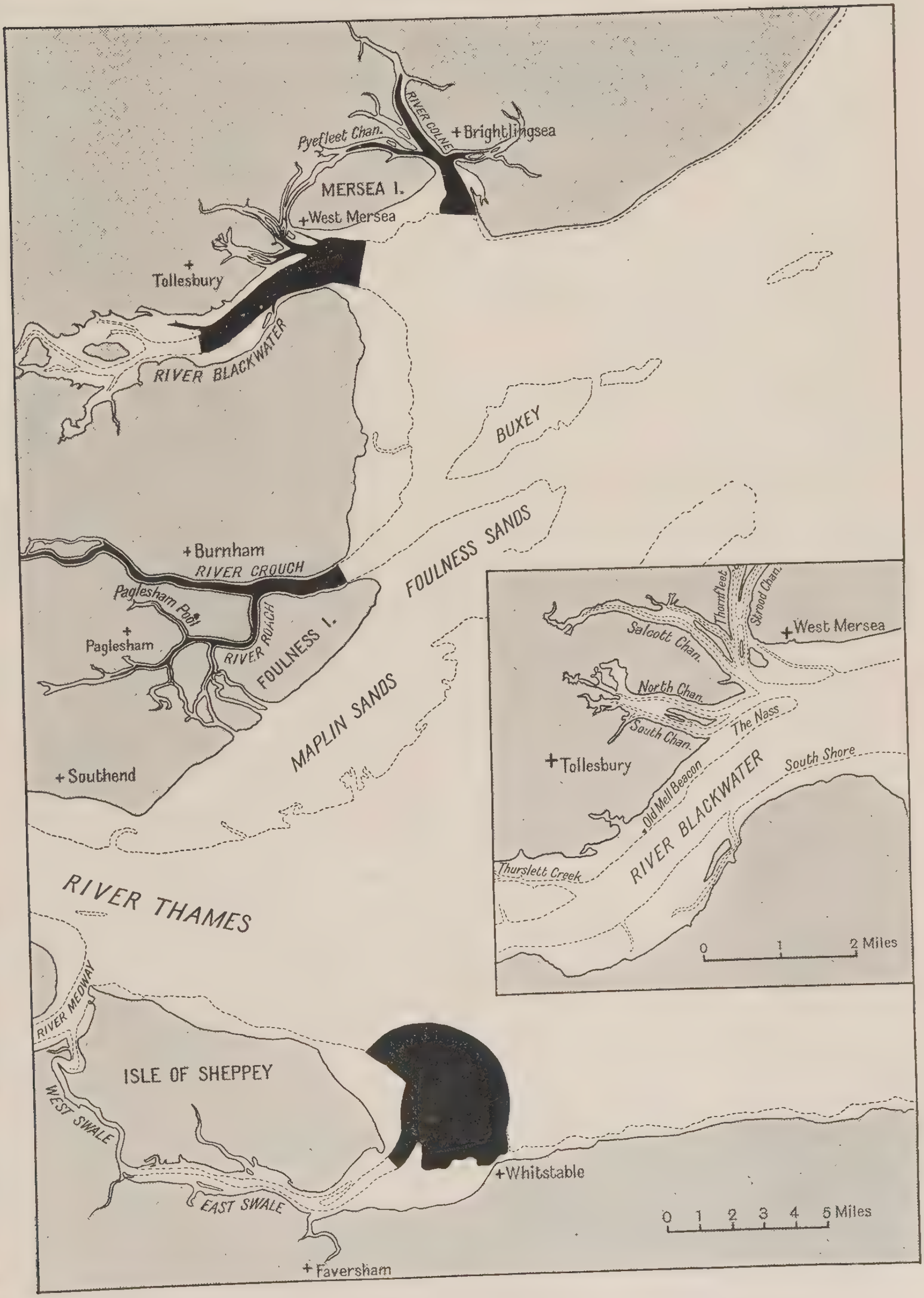

Pyefleet Chan.
RIVER COLNE
+ Brightlingsea
MERSEA I.
+ West Mersea
+ Tollesbury
RIVER BLACKWATER
BUXEY
FOULNESS SANDS
+ Burnham
RIVER CROUCH
Paglesham Pool
+ Paglesham
RIVER ROACH
FOULNESS I.
MAPLIN SANDS
+ Southend
RIVER THAMES
RIVER MEDWAY
ISLE OF SHEPPEY
WEST SWALE
EAST SWALE
+ Whitstable
+ Faversham
0 1 2 3 4 5 Miles
Thornfleet
Strood Chan.
+ West Mersea
Salcott Chan.
North Chan.
South Chan.
The Nass
+ Tollesbury
Old Mell Beacon
RIVER BLACKWATER
South Shore
Thurslett Creek
0 1 2 Miles

Fig. 1. Chart of the known distribution of *Urosalpinx cinerea* on British oyster beds (from Admiralty Chart). Inset—the Blackwater estuary.

and American oysters have in the past been laid down at several points on the east, south, and north-west coasts, e.g. Cleethorpes and the Orford River on the east, Poole Harbour, Emsworth and Bosham creeks, and the River Yealm on the south, and the Menai Straits in the north-west, while many years ago the beds at Hayling Island were used for wintering oysters from the east coast. If, therefore, as Orton & Winckworth (1928) think, *Urosalpinx* was introduced many years ago with *Crepidula*, an opinion with which the evidence collected by the present writer does not entirely agree, there is a good chance of it occurring wherever American oysters have been laid down. Since *Urosalpinx* lays its eggs in capsules from which the fully formed young tingles emerge, the larval stages being passed in the capsule, it is clear that to infect new localities tingles or their spawn must be carried there along with oysters or cultch.

Until the end of 1939 *Ocenebra erinacea* occurred in fair numbers along with *Urosalpinx* on the east coast oyster beds. Samples of tingles obtained from the River Blackwater in 1938 and 1939 included from 6 to 12 % of *Ocenebra*. Following the extremely cold winter of 1939–40 and the only slightly less cold winter of 1940–1, *Ocenebra* has disappeared from the Blackwater beds; during a fortnight's dredging in May 1941 not a single live specimen was encountered, although empty shells were common. Empty shells of *Urosalpinx* are, on the other hand, only occasionally found, and it seems that the 'great frost' of 1939–40 did not kill off any significant number of *Urosalpinx*; this is hardly surprising, for in its natural home on the Atlantic coast of the U.S.A. it is subjected to much greater extremes of cold and heat than are ever recorded here.

The progressive replacement of *Ocenebra* by *Urosalpinx* in the River Blackwater is well illustrated by some figures given by Orton & Lewis (1931). After the severe winter of 1929 *Ocenebra* was practically wiped out, while *Nucella* was much reduced in numbers—*Urosalpinx*, on the other hand, appeared actually to increase (Table I).

TABLE I. SHOWING THE PERCENTAGE OF *OCENEBRA*, *UROSALPINX* AND *NUCELLA* IN DREDGED CATCHES FROM THE RIVER BLACKWATER (FROM ORTON & LEWIS, 1931)

	Urosalpinx %	*Ocenebra* %	*Nucella* %	Total
1928	7·8	41·2	51	1739
1929	71·2	0·2	28·6	584
1930	77·9	0·11	22	5467

Dredged catches taken in 1941 contained only 1–5 % of *Nucella*, so that this species has also decreased markedly in recent years. During the period 1930–8 *Ocenebra* increased until in 1938 it formed 6–12 % of the catch; it may therefore once again re-establish itself in the Blackwater by migration or transport from outside.

In any river system *Urosalpinx* appears to favour the shallow creeks rather than the main river, and muddy bottoms rather than those of hard gravel or sand. The hard flats off Whitstable seem to be definitely less favourable than, for example, the muddy creeks at West Mersea. Federighi (1931) has shown that the range of salinity tolerated by *Urosalpinx* is much greater than that under which the cultivation of the European native oyster (*Ostrea edulis*) can be carried on.

Migration and movement generally in *Urosalpinx* has been studied in America by Federighi (1931) and Galtsoff *et al.* (1937) with somewhat conflicting results. Federighi, employing tagged drills planted on oyster bottoms at low water, found that even after one month the tagged drills had not moved more than 10–15 ft. from their original situation. It is stated that this was not due to the presence of unlimited food, for drills placed on a hard bottom 20 ft. from an oyster bed did not move towards it. Federighi notes also that contiguous oyster beds in Hampton Roads, Va., which had been left undisturbed for two years, were infested by drills in different quantities; he therefore concluded that significant migration does not take place. Experiments made by Galtsoff *et al.* (1937) did not confirm Federighi's conclusion. Marked drills were released on different types of bottom devoid of oysters and shells, and wire bags, some containing seed oysters and others containing only shells, were planted on the bed at different distances from the drills. In all cases more drills migrated to the bags containing seed oysters than to those containing no live oysters. These experiments were conducted over a period of several months and were thought to show definitely that the migration of drills was influenced by the position of food and that they tend to move towards it at the rate of at least 150 ft. in 48 hr. Galtsoff *et al.* therefore conclude that migration may play an important part in the distribution of drills. It is as well to remember, however, as these authors point out, that under natural conditions the behaviour of *Urosalpinx* is determined by the combined and sometimes antagonistic effects of several factors and cannot be attributed to a single cause.

There is a general belief among Essex oystermen that tingles are able to detect the presence of freshly laid oysters and to move on to them, and several instances were related of tingles which appeared suddenly on oysters laid down at low-water mark on ground previously free of this pest. This move is, however, observed during the breeding season, and it might be argued that the tingles moved on to the oysters from the surrounding clean bottom for the purpose of depositing their spawn. During 1941 a wooden trough 215 cm. long, coated inside with pitch and filled with sea water, was used at Conway to study the movements of marked drills. Food—spat or brood oysters, or barnacled stones—was placed at varying distances from the centre, towards one end of the trough, and the drills lined up at the centre and their movements noted at intervals of 30 min. or more. Ten drills were employed in most experiments and water temperatures were recorded. When the illumination over the whole surface of the trough was the same it was found that the drills moved at random and showed no ability to detect the presence of food or tendency to move towards it. This was true even when the distance between the drills and the food was reduced from the maximum of about 105 cm. to as little as 35 cm. These experiments were repeated a great number of times with identical results. Until steps were taken to even out the intensity of light by appropriate shading, the drills tended to move out of the sun in bright weather and take up a position on the shady side of the trough. Individual drills varied considerably in the degree and rate of their movements, but the maximum rate of creeping for a distance of not less than 90 cm. was 3 cm./min., at a temperature of 14·4° C. This compares favourably with the figure given by Federighi, 2·6–2·8 cm./min. at 26·5° C. This high rate of creeping was only observed once, and in the majority of experiments the most active drill covered the distance of 105 cm. to the end of the trough in 1–1½ hr., a rate of creeping of 1·17–1·75 cm./min. This rate was observed on occasions at all temperatures between 13 and 23° C. At temperatures from 23 to 25° C. only very slight movement occurred, above 25° C. none. Such high temperatures are probably never experienced on British oyster beds, but were frequently exceeded in the laboratory experiments of Federighi without apparently causing any inconvenience to the drills.

Drills moving consistently in one direction at the rate of 1·5 cm./min. would cover a distance of 43·2 m. in 48 hr. The rate of travel observed under natural conditions by Galtsoff *et al.* was 150 ft. (45·72 m.) in 48 hr. This was the minimum rate of travelling for the drills that reached the bags containing seed oysters, and presumed that they travelled in a straight line. It seems certain, therefore, that *Urosalpinx* is capable of moving at this rate; but it has not yet been satisfactorily established that it is able to detect the presence of food and move towards it, and it seems unlikely, from the limited evidence available, that migration is of more than local significance in the distribution of the species.

Growth

The shell dimension most useful in the study of growth rates in gastropods is the height from the tip of the siphonal canal to the top of the spire; this dimension has been employed in this study. In most localities in America the adult *Urosalpinx* is stated (Galtsoff *et al.* 1937) to average slightly over 2·5 cm. in height, but at Seaside, Va., specimens as large as 6 cm. in height are said to be very common. Federighi (1931) found that the largest females from Chesapeake Bay measured 3·3 cm. and the largest males 2·9 cm., but the most usual size was from 2·1 to 2·5 cm. The giants from Seaside, Va., appear to parallel the giant forms of *Nucella lapillus* described by Moore (1936) from the British Museum collection, having been obtained originally from Minehead and Swanage. The present writer has subsequently collected a number of such giants from the River Crouch. The peculiar environmental conditions leading to the production of these giant forms are not known, but in some gastropods parasitism is a factor of importance (Rothschild, 1936).

Urosalpinx on British oyster beds considerably exceeds the size generally reached in America. The largest individuals obtained were 4·3 cm. in height, and the only one sexed of this size was a female. The largest male measured 3·9 cm. Males over 3·5 cm. are always rare, but very large females are not uncommon on grounds which have not been worked intensively. This increase in size of an American form in British waters is also well shown by the slipper limpet, *Crepidula fornicata*.

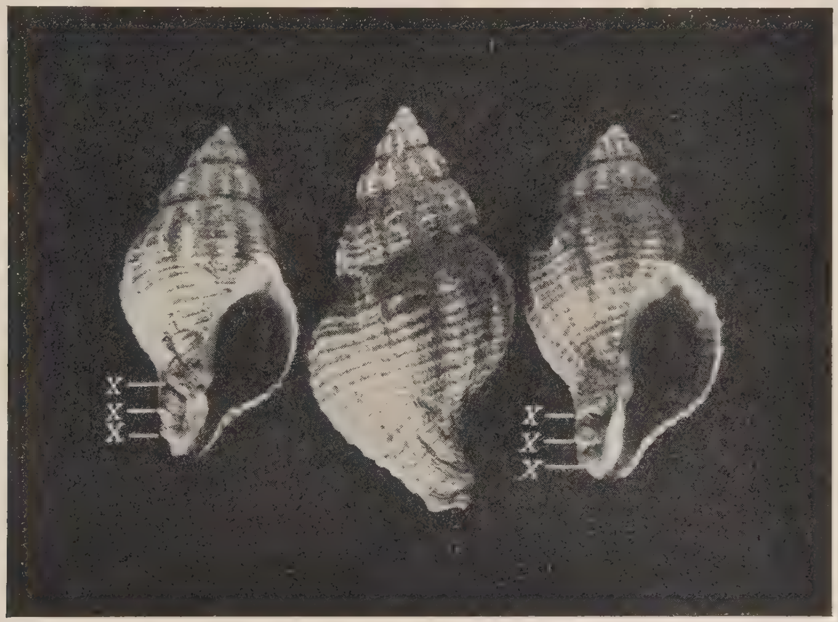


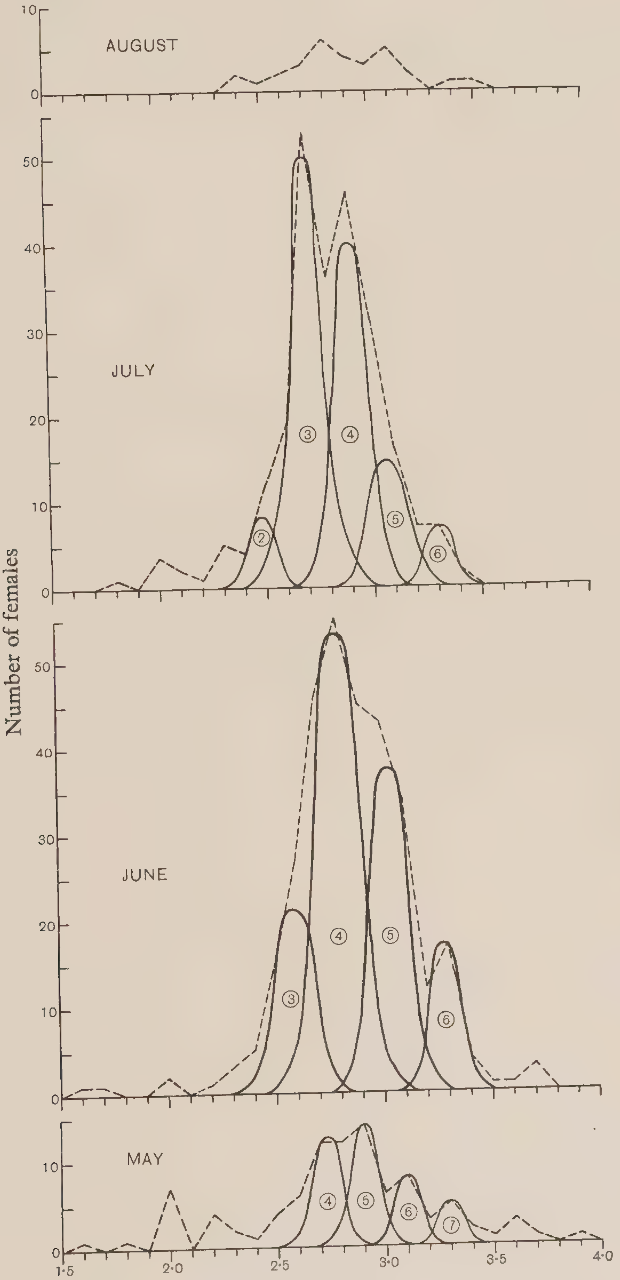
Fig. 2. *Urosalpinx cinerea* Say, showing growth marks (*X*) on the shell bounding the siphonal canal: × 1½.

Apart from the general observation that females grow larger than males the American work contains no information on growth rate or duration of life. This observation is equally true of *Urosalpinx* on British beds, and in the following analysis of growth males and females have been treated separately. Size-distribution curves of catches of females usually show a number of closely approximated peaks, and it appears that the annual increment in height is small after the first few years. This impression is confirmed by a study of the growth marks which may frequently be clearly seen on the tip of the shell bounding the siphonal canal (Fig. 2). Such growth marks in large shells are usually separated from the extreme tip and from each other by 0·1–0·3 cm. Considerable overlapping of successive year groups must therefore occur, and this seriously impedes or prevents altogether the fixing of the position of the peaks in the frequency curves without recourse to further analysis. For this reason wherever possible the analysis of size-distribution curves has been carried out by the freehand drawing method advocated by Buchanan-Wollaston & Hodgson (1929).

TABLE II. SIZE DISTRIBUTION OF *UROSALPINX* SAMPLES COLLECTED FROM THORNFLEET, RIVER BLACKWATER DURING 1941

Shell height cm.	24 May 1941		10 June 1941		8 July 1941		26 August 1941	
	Females	Males	Females	Males	Females	Males	Females	Males
1·6	1	—	1	—	—	—	—	—
1·7	—	—	1	—	—	1	—	—
1·8	1	1	—	—	1	—	—	—
1·9	—	1	—	—	—	1	—	—
2·0	7	1	2	1	3	1	—	—
2·1	—	2	—	2	2	1	—	1
2·2	4	1	1	—	1	2	—	—
2·3	2	—	3	3	5	1	—	—
2·4	1	1	5	2	4	3	2	—
2·5	4	3	15	14	12	5	1	3
2·6	6	9	27	22	19	8	2	2
2·7	12	5	46	27	53	13	3	4
2·8	12	13	55	24	36	23	6	6
2·9	14	9	45	32	46	17	4	4
3·0	6	17	43	24	31	21	3	6
3·1	8	7	34	16	16	9	5	4
3·2	3	3	12	5	7	7	2	3
3·3	5	2	17	4	7	3	—	—
3·4	2	1	4	—	2	3	1	1
3·5	1	—	1	—	—	—	1	—
3·6	3	1	1	—	—	—	—	—
3·7	1	—	3	—	—	—	—	—
3·8	—	—	—	—	—	—	—	—
3·9	1	—	—	—	—	—	—	—

During 1941 a series of four samples of tingles was collected from the beach in Thornfleet (a creek running into the River Blackwater, Essex, near its mouth) at approximately monthly intervals from May to August (Table II). The first lot was collected on 24 May, when tingles were first appearing on the shore at the beginning of the spawning season, and the last on 26 August, when many had already migrated to deeper water. On each occasion all visible tingles were hand collected from stones, old drain pipes, mooring chains, etc., in the region of low-water mark of spring tides. On the same day that the first shore sample was collected a further lot was obtained by dredging in the channel adjacent to the beach. In Fig. 3 the size distribution of the females contained in the four monthly samples from Thornfleet are shown, those of May, June, and July being resolved into their components. The August sample was too small to permit of such treatment, while the May sample contained also those females dredged in Thornfleet on the same day. It will be seen that the modes of successive year groups are separated by an interval of about 0·2 cm., or slightly more in the younger age groups, and rather less in the older ones.



Height of shell in cm.

Fig. 3. Size-distribution curves of females from samples of *Urosalpinx* hand collected from the beach of Thornfleet, River Blackwater during 1941. The May sample also includes those dredged in the adjacent channel on the same day. In this and subsequent figures the numbers in the circles represent the probable age of each year group.

The comparison of the May and July groups shows the growth during 1941, up to 8 July, to have been rather less than 0·2 cm., as would be expected.

Comparison of the May and July samples shows the presence in July of two modes on the left-hand side not represented in May. In July these modes lie at 2·47 and 2·7 cm. respectively; but the sample also contains tingles as small as 1·8 cm. which cannot belong to the age group with the mode at 2·47 cm. The latter mode therefore must belong to the 2-year-old group, while the smaller tingles would belong to the 1-year-old class with a mode somewhere in the region of 2·0 cm. This 1-year-old group, which must numerically be the strongest, is very poorly represented on the shore during the summer.

Tingles hatched from egg capsules in July, and reared on small oyster spat in a plunger jar at Conway during 1941, reached a maximum height of 1·2 cm. by the end of the feeding period, the mode of the group being at approximately 1·0 cm. These tingles would start the following season at this size and might be expected to reach about 2·0 cm. by July, i.e. by the end of their first year. Dredged samples of tingles collected during the summer which contain fair numbers of very small tingles usually have a suggestion of a peak around this figure; unfortunately, as yet no method has been devised for collecting adequate samples of these small tingles, the majority of which pass through the rings of an ordinary oyster dredge.

That a growth rate similar to that observed in 1941 prevailed among female tingles in other years is shown by Figs. 4 and 5, which show the results of

TABLE III. SIZE DISTRIBUTION OF *UROSALPINX*

Shell height cm.	River Blackwater 15 July 1939		Thurslett Creek R. Blackwater 15 May 1940	River Roach 13 May 1939
	Females	Males	Females	Females
1·6	—	—	—	1
1·7	—	—	—	3
1·8	—	—	—	2
1·9	—	—	—	7
2·0	—	—	—	8
2·1	2	2	1	4
2·2	1	2	1	7
2·3	4	6	1	5
2·4	7	4	1	1
2·5	11	7	4	13
2·6	14	7	11	12
2·7	23	14	16	15
2·8	17	9	27	19
2·9	16	9	23	13
3·0	12	5	30	9
3·1	9	2	21	14
3·2	2	1	38	7
3·3	7	—	23	14
3·4	1	—	13	8
3·5	2	—	19	3
3·6	1	—	5	2
3·7	—	—	8	—
3·8	—	—	1	3
3·9	—	—	1	2
4·0	—	—	3	1

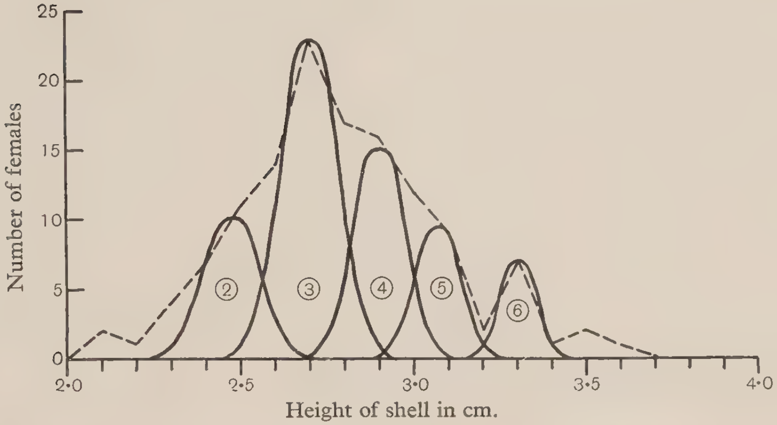


Fig. 4. Size-distribution curves of females from a sample of *Urosalpinx* dredged from the River Blackwater on 15 July 1939.

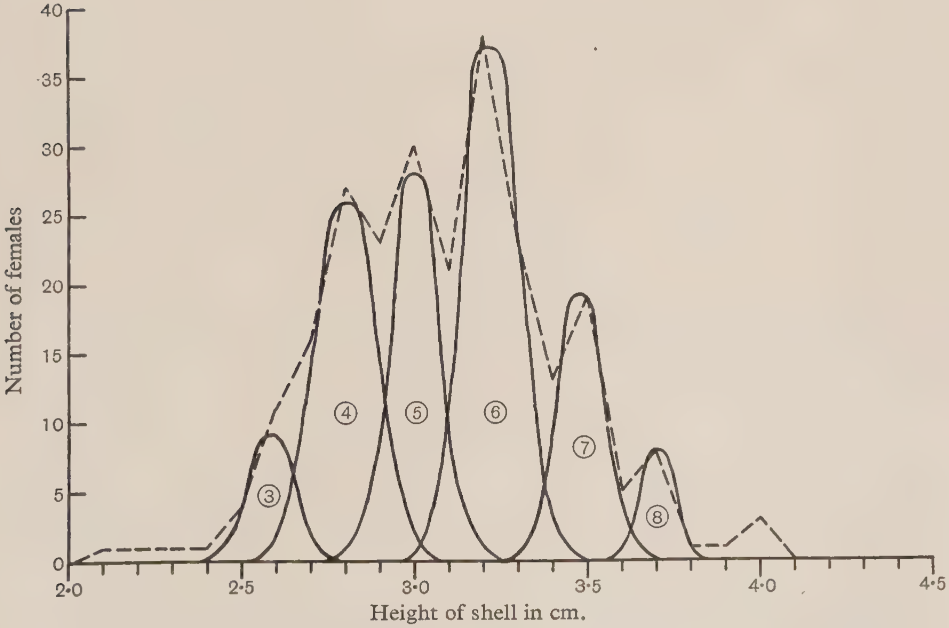


Fig. 5. Size-distribution curves of females from a sample of *Urosalpinx* dredged from Thurslett Creek, River Blackwater, on 15 May 1940.

analysing samples of tingles (Table III) dredged from the River Blackwater in 1939 and 1940. Both samples show a series of successive modes at intervals of about 0·2 cm., the position of the modes agreeing reasonably well with the corresponding sample of the year 1941, when due allowance is made for the lateness of the season in that year.

Although a number of samples of tingles have been collected from other rivers in Essex, only one (included in Table III) contains sufficient females to permit of the resolution of the frequency curve into its components. This sample was obtained in 1939 from the River Roach, a tributary of the Crouch, and is shown in Fig. 6. Successive modes are rather more distant than in the Blackwater collections, being approximately 0·25 cm. apart, indicating a more rapid growth rate in the River Roach. At least seven age groups are represented in this sample, the first mode representing most probably the 3-year-old group (cf. Fig. 2).

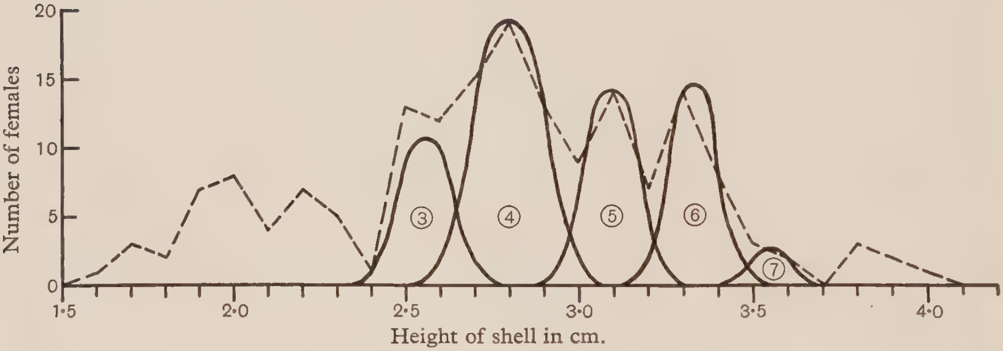


Fig. 6. Size-distribution curves of females from a sample of *Urosalpinx* dredged from the River Roach on 13 May 1939.

Summarizing the information obtained concerning growth rate in female tingles, from both size-frequency curves and the study of growth marks, it seems probable that in July, when a new batch of young emerges from the capsules, the 1-year-old group would average about 2·0 cm. in height, the 2-year-old 2·5 cm., 3-year-old 2·7 cm., 4-year-old 2·9 cm., 5-year-old 3·1 cm., 6-year-old 3·3 cm., 7-year-old 3·5 cm., 8-year-old 3·65 cm., 9-year-old 3·8 cm., 10-year-old 3·9 cm., and thereafter successive age groups would occur at intervals of 0·1 up to 4·3 cm., the largest size recorded. It seems probable that this 4·3 cm. specimen was about 14 years old.

Frequency curves derived from catches of male tingles obtained from Thornfleet in 1941 (see Table II) when analysed show successive modes at intervals of 0·2 cm. or rather less (Fig. 7). The July sample contains representatives of six age groups without taking into account tingles below 2·3 cm. in height. As in the females from these catches, the July sample shows two modes not present in May, so that the first mode in May must comprise indi-

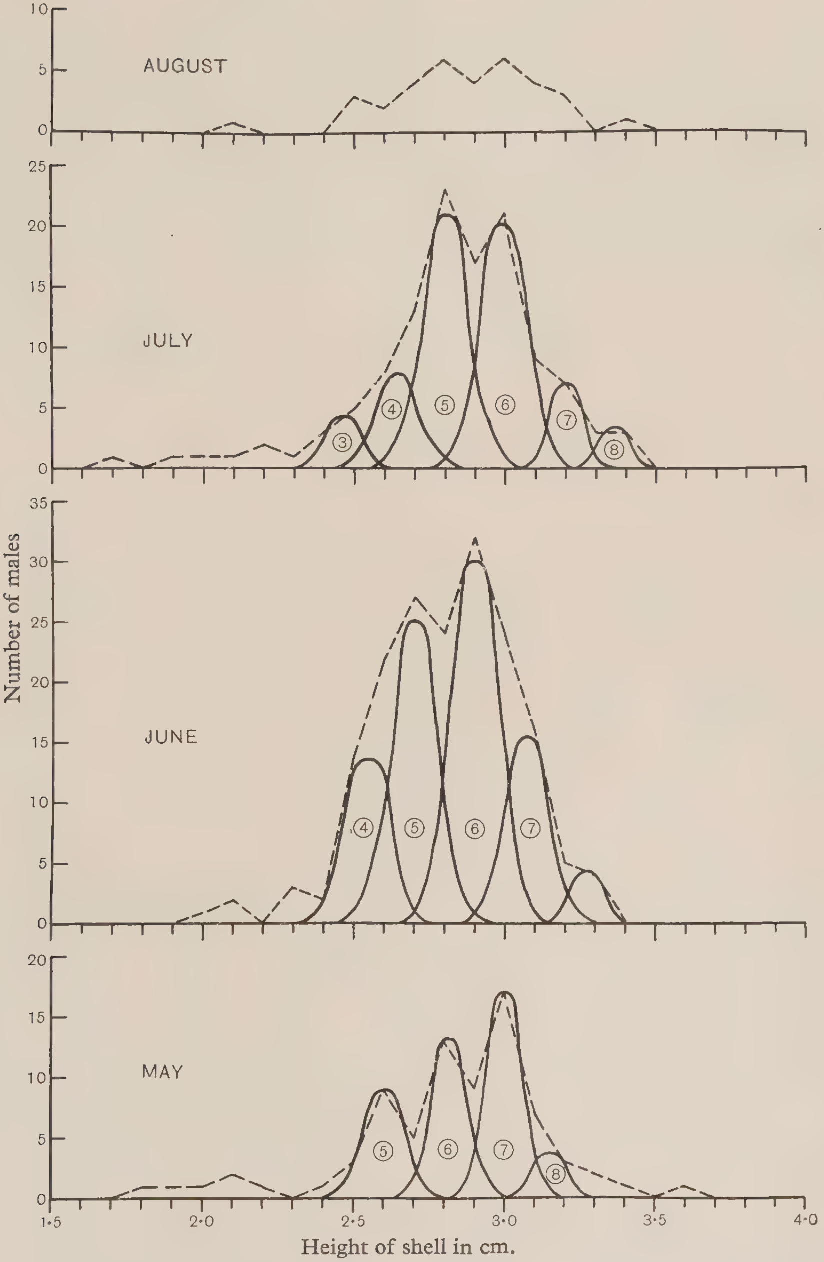


Fig. 7. Size-distribution curves of males from samples of *Urosalpinx* hand collected from the beach of Thornfleet, River Blackwater, during 1941. The May sample also includes those dredged in the adjacent channel on the same day.

viduals at least 3 years old; evidence presented below shows that they are in fact older. The July sample contains tingles down to 1·7 cm. in height which cannot belong to a group with a mode of 2·46 cm., consequently the latter must consist of individuals at least 2 years old. Comparison of Figs. 7 and 8, the latter being the analysis of a sample taken in July 1939 from the River Blackwater (see Table III), reveals an extra mode at 2·3 cm. in the 1939 sample in addition to one at 2·51 cm. corresponding to the first mode (2·46 cm.) in the July 1941 sample. When it is remembered that the 1941 season was very late the slight discrepancy between the position of the modes in the two years is explained. The July 1941 frequency curve (Fig. 7) would therefore, if complete, show a further mode slightly below 2·3 cm., but this age group could not reasonably contain individuals as small as 1·7 cm., the smallest size included in the sample, which must belong to a still earlier group. We therefore conclude that the first mode shown by the July 1941 sample (Fig. 7) represents the 3-year-old group and consequently the first mode in May 1941 represents the 5-year-old tingles.

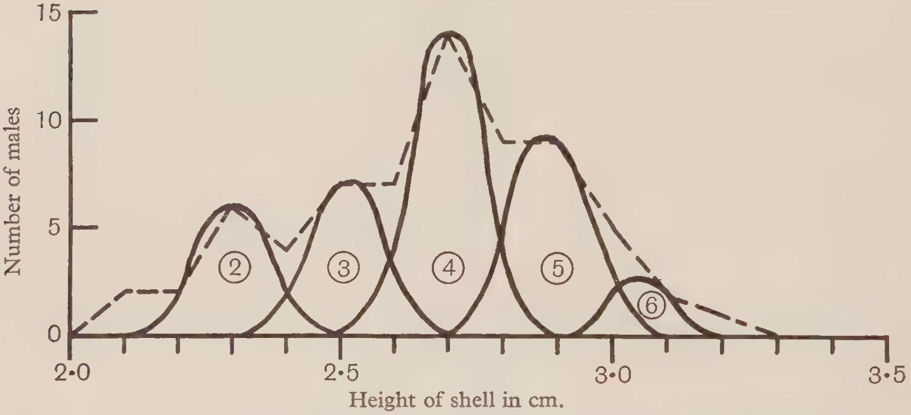


Fig. 8. Size-distribution curves of males from a sample of *Urosalpinx* dredged from the River Blackwater, 15 July 1939.

In males growth thus proceeds roughly as follows: 1st summer, 1·0 cm.; 1st year, 1·0–1·8 cm.; 2nd year, 1·8–2·3 cm.; 3rd year, 2·3–2·5 cm.; 4th year, 2·5–2·7 cm.; 5th year, 2·7–2·9 cm.; 6th year, 2·9–3·1 cm.; 7th year, 3·1–3·25 cm.; 8th year, 3·25–3·4 cm.; 9th year, 3·4–3·55 cm.; 10th year, 3·55–3·65 cm.; and thereafter at about 0·1 cm. per annum. The largest male so far collected, viz. 3·9 cm., would therefore be about 13 years old, a similar age to that deduced for the largest female. Although both males and females up to this extreme age may be found on occasion, no substantial number of males over 3·6 cm. or females over 3·9 cm. have been collected, so that the approximate duration of life in *Urosalpinx* is 10 years.

PARASITES

During the examination of dredged samples of *Urosalpinx* for sex seven specimens have been found containing what appears to be a very specialized arthropod parasite, probably an isopod, in the liver region. All the tingles so affected came from the oyster beds in the mouth of the River Blackwater. In addition one individual from the same river has been found with the gonad completely riddled with rediae containing cercariae agreeing exactly with *Cercaria sensifera*, which Stunkard & Shaw (1931) have described in *Urosalpinx* from Woods Hole, U.S.A. These authors point out that it is possible that *Cercaria sensifera* is identical with *C. purpurae* (Lebour, 1912), found in *Nucella lapillus* in British waters, although the survival and establishment of an alien trematode is by no means impossible.

BREEDING

As in several other carnivorous gastropods the eggs of *Urosalpinx* are deposited in tough horny capsules each of which contains several eggs. The capsules are attached to the substratum in clusters and from them fully formed young tingles emerge. The egg capsules have been described and compared with those of *Nucella* and *Ocenebra* by Orton & Amirthalingam (1929), while the actual method of formation of the egg capsule and the structure of the organs involved has been studied by Fretter (1941) in a variety of British stenoglossan gastropods, including *Ocenebra* and *Nucella*. The deposition of egg capsules usually begins during late April or early May and continues during June and July. Spawning reaches its maximum during June and thereafter declines. During 1940 the first few capsules were found in the River Blackwater in the second week in April, but although the winter had been exceptionally severe the spring was milder than usual. In 1941, when the spring months were exceptionally cold, no spawn was found until the third week of May. During the last fortnight in May, when the water temperature rose steadily from 11·6 to 13·4° C., the number of tingles dredged in the act of spawning and the total amount of spawn caught per day increased steadily. It seems probable therefore that spawning begins each year in British waters when the sea temperature in its seasonal rise reaches 12–13° C. This estimate agrees well with the figure given by Galtsoff *et al.* (1937) for Delaware Bay, U.S.A., where capsule deposition is stated to begin when the water temperature has reached 13·9° C. The bulk of the spawn is deposited during May and June; but small numbers of freshly laid capsules may be found in August, e.g. a sample of spawn collected from the River Blackwater on 27 August 1940 contained about a dozen freshly deposited capsules—a small number contained embryos in early stages of development, but the great majority of the capsules had already hatched or were in process of hatching. The earliest date at which capsules have been seen from which the young tingles had emerged is 2 July (1940); as already mentioned, the first few capsules laid in that year were seen during the second week in April. Several batches of spawn obtained in June 1940 showed no embryos advanced beyond the early shelled stages. In this year, although a few capsules were seen in April no substantial quantity was deposited until May. We may therefore conclude that incubation of capsules under natural conditions occupies about two months.

Further observations made in 1941 confirm the above estimate. Deposition of capsules began in the Blackwater during the third week in May, spawning being in full swing by the first week in June. No hatching had occurred in samples obtained in June, no capsules containing embryonic stages later than morulae; but samples of spawn procured on 23 July showed a large number of capsules with young tingles emerging. The period of incubation was therefore 7–8 weeks.

In the laboratory, incubation of capsules deposited by tingles in experimental tanks at Conway has been carried out in plunger jars, with and without frequent changes of water. The periods of incubation observed were 27–32 days at an average temperature of 22·6° C., and 44–50 days at an average temperature of 18·3° C. According to the records given by Orton & Lewis (1931) of sea temperature in the River Blackwater during the four years 1926–8 and 1930, the mean during the period mid-May to the end of August, when the spawn of *Urosalpinx* is being incubated, varies between 13·5 and 19° C., so that the period of incubation under natural conditions in this river might be expected to exceed slightly the period of 44–50 days recorded above. This expectation accords very well with the period of about two months deduced from observations of the time of commencement of spawning and hatching. In years when the water temperature rises rapidly during spring, spawning may begin in April, but it is not usually general until May or, in late seasons such as 1941, until June. Emergence of young tingles therefore generally occurs about the middle of July.

Federighi (1931) found that the average period of incubation under laboratory conditions of *Urosalpinx* capsules from Hampton Roads, Va., U.S.A., varied between 36 and 44 days with a mean of about 40 days. Water temperature in these experiments varied from 22 to 28° C. Galtsoff *et al.* (1937) give the period of incubation in Delaware Bay as varying from 21 to 53 days. In American waters spawning of *Urosalpinx* apparently occurs throughout the summer and autumn in many localities: e.g. in Delaware Bay it is stated that spawning begins early in April when the water temperature reaches about 14° C. and continues until late November. In Cape Cod waters two separate periods of spawning were observed, the second occurring during the second half of September. There was no evidence until 1941 that a second period of spawning ever occurred in this country, but in that year tingles kept in a tank at Conway spawned intermittently during late August and September, the last capsules being deposited on 30 September. During the latter part of this period water temperature fluctuated between 13 and 17° C. During

September groups of 11, 10, 12 and 14 capsules were laid, all of which, as will be seen later, are less than the usual number deposited in one laying early in the season.

Not a great many opportunities have occurred of noting the number of capsules deposited by a single tingle at one laying, but about a dozen such groups which have been noted during the normal spawning season have ranged from 20 to 35, with an average of 25 per female. This is in fair agreement with the work of Federighi (1931) in America, who found that an average of 28 capsules was deposited by isolated females. Galtsoff *et al.* (1937) state that in Delaware Bay females may deposit as many as 50 capsules, which are grouped in clusters. It is possible therefore that the groups noted above, averaging 25 capsules, may form but a part of the total production of the drills concerned. In practice two or more drills are frequently found spawning together on the same shell, while a large stone or pile of old chain at or about low-water mark may harbour a dozen or more spawning drills. This does not indicate, I think, a social habit among drills but merely the close occupation of the available satisfactory spawning sites. Capsules are deposited at the rate of three or four per day. Orton (1930) states that there is an inshore migration of *Urosalpinx* in spring and early summer, and that the species spawns heavily in shallow water. This is very noticeable in the river Blackwater, but very large numbers spawn also at all depths, and the selection of a spawning site seems to be governed mainly by the presence of abundant 'clocks', stones, or other firm objects, rising well clear of the bottom and offering situations free from silt. *Urosalpinx* kept in a tank at Conway deposited capsules most frequently on the vertical sides of the tank or in situations which were overhung.

On American oyster beds the average number of embryos per capsule is stated to be 9 in Hampton Roads, Va., the numbers varying from 3 to 20, and 8 in Delaware Bay, varying from 0 to 20. On British beds *Urosalpinx* is more prolific, for 1423 capsules from different localities averaged 11·74 embryos per capsule; of this total 823 capsules contained early stages and 600 contained shelled veligers practically ready to hatch. The early stages averaged 12·47 embryos per capsule and the shelled stages 10·74 per capsule. There is therefore a mortality during incubation of 1·73 embryos per capsule, or 13·9%. This figure is much lower than that of Federighi (1931), who found only an average of 5·1 larvae emerging per capsule (the average number of eggs being 8·8)—a loss of 42%. The number of embryos per capsule may vary from 1 to 29 in this country, but in capsules from one parent there is little variation.

If the average number of capsules laid per female in this country is assumed to be 25, from each of which on the average 10·74 drills emerge, then each female drill gives rise to 268·5 young. It is probable that the actual figure is somewhat higher, as Galtsoff *et al.* (1937) have shown that after depositing one group of capsules females may move away and later deposit further batches. It seems likely therefore that from the capsules laid by one female on British beds not less than 300 young drills emerge.

In order to determine at what age and height of shell deposition of egg capsules begins, 21 tingles varying from 1·8 to 2·4 cm. in height were placed in a floating wooden wire-covered cage in one of the tanks at Conway towards the end of June 1941 and a daily examination made for egg capsules. Barnacles were provided as food. Thirteen of the 21 small tingles survived the summer and only three were noted to deposit egg capsules. The largest group of capsules deposited numbered only 13, the others being considerably smaller. Of the 13 tingles remaining at the end of the summer only three were females; these were the largest of the group and were almost certainly 2 years old. The result of this experiment was inconclusive, but suggests that 2-year-old females below 2·5 cm. in height at the beginning of the season deposit a small number of egg capsules. In other lots of tingles two individuals measuring 2·4 cm. and one measuring 2·5 cm. have been noticed in the act of spawning; each of these laid only a small number of capsules in late summer. On the other hand, during May 1941, when breeding was just beginning, it was noticed that the majority of the females 2·2 cm. or less in height had undeveloped gonads and accessory reproductive organs and appeared to be definitely immature. It seems therefore that some of the largest of the tingles below 2·5 cm. in height in July and August, which are probably 2 years old, or possibly unusually small 3-year-olds (see Fig. 2), do spawn to a slight extent, but it is highly probable that the amount of spawn deposited is insignificant in relation to the production of older tingles. The question is of some practical importance, since such small tingles are only retained in very small numbers by the dredge and at present there is no other practicable method of removing them from the beds below low-water mark.

Among the numerous very large females examined only two or three have been found with the gonad in an exhausted condition. None of these was below 4·1 cm. and one was 4·3 cm. in height, the largest size yet recorded; on the other hand, individuals up to 4·1 cm. have been observed in the act of depositing capsules. The latter could not be less than 10–12 years old, so that the reproductive life of each female may extend over 7 years (ignoring the first 2 years when some capsules may be deposited), in each of which she may give rise to 300 young.

It is very noticeable from Fig. 3 how the dominant age group changes during the season among the female tingles collected from the beach in Thornfleet, River Blackwater. In Fig. 9 the composite May sample shown in Fig. 3, which is made up of two separate catches, the one dredged and the other hand collected on the same day, is split up into its components (see Table IV), males and females being graphed separately. The females in the shore sample are seen to belong principally to one year group, which by comparison with Fig. 3 is seen to be the 4-year-old class, with smaller numbers of 3- and 5-year-old tingles. The sample dredged on the same day in the channel adjacent to the beach shows that 5-, 6-, and 7-year-old tingles were also present in numbers in the creek as well as of course large numbers of 1-, 2-, and 3-year-old females which would not be picked up in quantity by the dredge; but the first tingles to come to shore for spawning were mainly 4 years old. The males accompanying these females belong mainly to the 5- and 6-year-old groups (see Fig. 7), with a few younger individuals, but the dredged sample shows that numerous older drills remain in the channel.

In June the 4-year-old females (mode at 2·8 cm.) are dominant on the shore with comparatively small numbers of 3-year-olds and a few smaller tingles, but a month later, in July, the 3-year-olds have enormously increased and form the dominant group, although older tingles are also fully represented, while there are a few 2-year-old and even younger tingles. Among males the tendency is similar, for as the season advances the younger age groups appear on the shore in increasing numbers, but the proportion of 1- to 4-year-old males is never commensurate with their numerical strength on the beds.

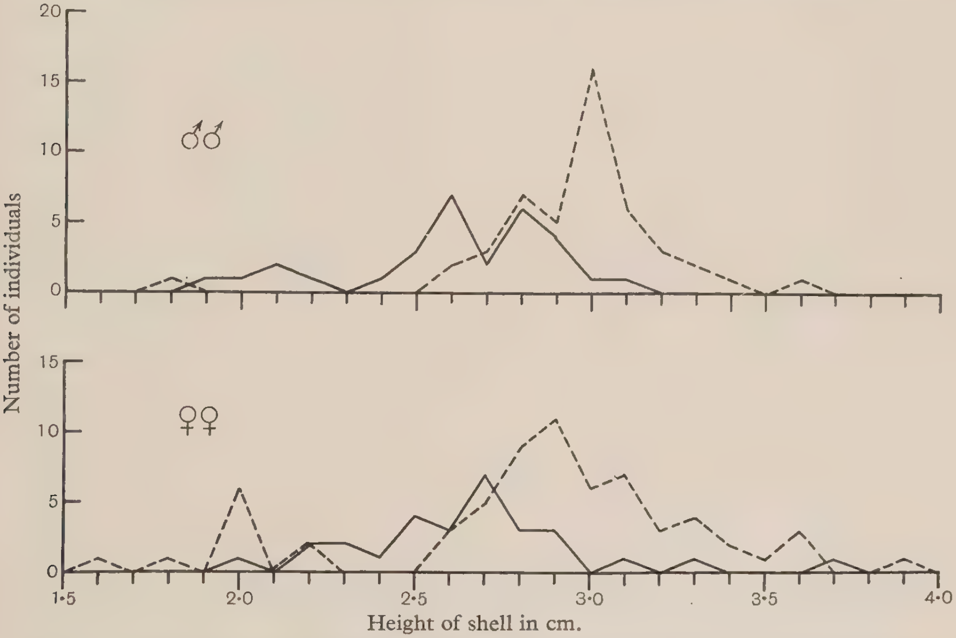


Fig. 9. Comparison of hand-collected (continuous line) and dredged (pecked line) samples of tingles collected on the same day (24 May 1941) from Thornfleet, River Blackwater.

It should be remembered that these samples from Thornfleet are hand collected and therefore representative of the population on the beach at the time. The females have moved inshore primarily for the purpose of spawning, and it appears therefore that the 3-year-old females spawn about a month after the older groups, while only a few of the 2-year-old and smaller tingles migrate inshore. It has been noted that when such small tingles were isolated some deposited egg capsules, but each drill produced only a fraction of the usual number laid by mature individuals. These facts, taken in conjunction with the absence of significant numbers of 1- and 2-year-old females from the spawning population on the beaches, tend to prove that full spawning does not occur until the tingles are 3 years old.

Table IV. Size Distribution of Dredged and Hand-collected Samples of *Urosalpinx* from Thornfleet, 24 May 1941

Shell height cm.	Dredged		Hand-collected	
	Females	Males	Females	Males
1·6	1	—	—	—
1·7	—	—	—	—
1·8	1	1	—	—
1·9	—	—	—	1
2·0	6	—	1	1
2·1	—	—	—	2
2·2	2	—	2	1
2·3	—	—	2	—
2·4	—	—	1	1
2·5	—	—	4	3
2·6	3	2	3	7
2·7	5	3	7	2
2·8	9	7	3	6
2·9	11	5	3	4
3·0	6	16	—	1
3·1	7	6	1	1
3·2	3	3	—	—
3·3	4	2	1	—
3·4	2	1	—	—
3·5	1	—	—	—
3·6	3	1	—	—
3·7	—	—	1	—
3·8	—	—	—	—
3·9	1	—	—	—

Sex Ratio

American work gives no indication of any abnormality in the sex ratio of catches of *Urosalpinx*. It has already been recorded, however (Cole, 1941), that the sex ratio in dredged catches from British oyster beds shows some striking peculiarities. Table V gives the proportions of the two sexes in all dredged catches of *Urosalpinx* obtained from the River Blackwater during the years 1938–41. Allowance should be made when studying this table for the fact that the 1941 season was characterized by an abnormally cold spring, the season being reckoned as roughly a month later than usual on the Blackwater. Bearing this in mind it is clear that, generally speaking, males and females are equally abundant in dredged catches of tingles at the outset of the season, but thereafter the proportion of females rapidly increases reaching a peak of over 90% during June and declining again in July and August to approximately 50%. It will be noted also that the seasonal curve of percentage of females follows very closely the intensity of spawning. This suggests that as the females move about and take up their spawning positions they become more

Table V. Dredged Catches of *Urosalpinx* from the River Blackwater

Date	Locality	Females	Males	Total	% females
7. iv. 38	R. Blackwater	56	55	111	50·5
10. iv. 40	Thornfleet, R. Blackwater	13	13	26	50·0
2. v. 40	Thornfleet, R. Blackwater	82	58	140	58·1
8. v. 40	Thurslett Creek, R. Blackwater	124	19	143	86·7
15. v. 39	Thurslett Creek, R. Blackwater	55	13	68	80·9
15. v. 40	Thurslett Creek, R. Blackwater	246	33	279	88·2
15. v. 39	South Shore, R. Blackwater	69	28	97	71·1
19. v. 41–27. v. 41	Thurslett—Old Mell Beacon, R. Blackwater	213	75	288	74·0
20. v. 41–29. v. 41	S. Shore, R. Blackwater	58	24	82	70·7
21. v. 41	Nass End, R. Blackwater	65	61	126	51·6
24. v. 41	Thornfleet, R. Blackwater	65	47	112	58·0
10. vi. 41	Thurslett Creek, R. Blackwater	84	13	97	86·6
13. vi. 40	R. Blackwater	57	3	60	95·0
17. vi. 41	Thurslett—Old Mell Beacon, R. Blackwater	112	7	119	93·1
24. vi. 41	Thurslett—Old Mell Beacon, R. Blackwater	149	22	171	87·2
2. vii. 40	R. Blackwater	81	30	111	73·0
15. vii. 39	R. Blackwater	129	68	197	65·5
26. viii. 40	R. Blackwater	92	68	160	57·5

susceptible to capture by an oyster dredge, while the males remain in positions where the dredge rarely captures them. It is known (see Federighi, 1931) that females tend to crawl up on to objects raised off the sea bottom to deposit their spawn, and in these situations they may be particularly liable to capture; but it is highly probable that on most grounds the scraping bar of the dredge digs slightly into the bottom, for the contents of a dredge bag usually includes some mud and a good deal of fine gravel and shell fragments. Nevertheless, female tingles upon objects slightly raised off the bottom may be reasonably considered as particularly liable to be included in a dredge bag; it is therefore likely that the proportion included will increase as the intensity of spawning increases and more females take up spawning positions. It is curious, however, that the males are not with the females or at least crawling among the cultch in search of food. Such evidence as we possess concerning the behaviour of the male tingles does in fact suggest that they are to be found with the spawning females. A sample hand collected on 24 May 1941 from stones, old drain pipes, mooring chains, etc., on the beach at low-water mark in Thornfleet, River Blackwater, includes practically equal numbers of males and females (Table VI), showing that at the beginning of the spawning season males are also on the move and may be found on raised objects alongside the females. The dredged catch from the channel adjacent to the beach, collected on the same day (see Table V) contained only 42% of males. Slightly later in the year at the height of the spawning season similar collections off the same raised objects in Thornfleet gave a preponderance of females (see Table VI), but males were also very numerous, and the percentage of females did not approach the high figures shown by dredged catches at this season. At the end of

Table VI. Hand-collected Samples of *Urosalpinx* from Thornfleet, River Blackwater

Date	Females	Males	Total	% females
24. v. 41	29	30	59	49·2
10. vi. 41	316	176	492	64·2
20. vi. 40	33	23	56	58·9
8. vii. 41	245	119	364	67·3
26. viii. 41	30	34	64	47·0

August another collection from the same raised objects gave approximately equal numbers of males and females. These hand-collected samples show that males are to be found along with spawning females on raised objects throughout the breeding season, at least on inshore grounds; it is all the more remarkable therefore that dredged catches from offshore contain so few males at this season. The difference in size between males and females of the same age will lead to some selection of females by the dredge, for it is clear from the size-distribution curves of dredged catches (e.g. that shown in Fig. 4) that drills below about 2·7 cm. are particularly liable to be lost through the meshes of the dredge bag. Thus the first three year classes of males with modes at *ca.* 1·8, 2·3, and 2·5 cm. at midsummer are likely to be missed, whereas only the first two year classes of females with modes at *ca.* 2·0 and 2·5 cm. are equally liable to pass through the meshes.

The continued collection of large numbers of females and small numbers of males during normal dredging would, in a population with a normal sex ratio, bring about a considerable alteration in the proportion of the two sexes in that population within a few years. For this reason on well-worked grounds such as those in Thurslett Creek, River Blackwater, one might expect to find, at least occasionally, a preponderance of males, yet in the nineteen dredged samples listed in Table V the highest proportion of males is 50%. In a previous note on this question (Cole, 1941) I have mentioned the possibility that sex change in a proportion of the population might be responsible for the observed abnormalities in the sex ratio. There is, however, no evidence whatever to support this hypothesis During 1941, 20 drills caught in the act of spawning were isolated during the summer and examined in October; 19 were female and one had died. Careful search has been made among thousands of drills for individuals with characters intermediate between those of males and females, but none has been found, yet the sexes are easily recognizable and the penis is quite a large organ, so that if variations occurred in this organ they would be easily detected. The inference to be drawn is that sex change does not occur.

Feeding

It is extremely difficult to evaluate the destructiveness of tingles, since it is impossible to determine the number of very small oysters killed on the beds. By keeping tingles enclosed with oysters of various sizes some idea of the potential damage may, however, be obtained. This has been done at Conway during the summers of 1940 and 1941. Known numbers of tingles have been enclosed in wooden wire-covered floating cages with ample supplies of either 1- or 2-year-old oysters, and daily counts made of the quantity destroyed. Maximum and minimum water temperatures were also recorded. Drilling may begin as soon as the water temperature exceeds 11–12° C.; this usually occurs during April or early May, but very little feeding occurs at this time as the drills are actively spawning. In June, when the peak of the spawning season has been passed, intensive feeding begins and continues until the water temperature again drops below 11–12° C., usually towards the end of October.

TABLE VII. RATE OF DESTRUCTION OF SPAT AND BROOD OYSTERS BY *UROSALPINX*

Type of oyster	Year	No. of tingles	Oysters destroyed	Period of feeding days	Rate per day per tingle
1-year-old spat	1940	22	701	96	0·332
,, ,,	1940	36	173	10	0·481
,, ,,	1941	39	2168	127	0·438
2-year-old brood	1940	12	45	109	0·034
,, ,,	1941	30	92	98	0·032

In Table VII are given the rates of destruction of 1-year-old spat and 2-year-old brood oysters recorded during 1940 and 1941. For these experiments mixed lots of tingles varying in age from 3 to about 8 years were employed, all collected originally from the River Blackwater. In Fig. 10 the daily rate of destruction of 1-year-old spat in 1941 is correlated with the average daily water temperature. It will be noted from Table VIII that the rate of destruction of spat during July and August is considerably above the final average figure for what was practically the whole feeding period. It is very noticeable how the rate of feeding fell away immediately the water temperature dropped below 14° C. This figure may be regarded as the minimum at which full feeding activity begins. There was no close correlation between water temperature and rate of feeding until mid-July when the breeding season was practically over. During the short period between 22 July and 6 August spat were destroyed at the rate of 0·90 per tingle per day. There is a fairly good general correlation between water temperature and rate of feeding from mid-July onwards, but it is apparent that there is also a seasonal rhythm to some extent overriding the effect of temperature; tingles feed voraciously immediately after the completion of spawning, but thereafter a general decline in the rate of feeding sets in, although it rises and falls also in response to changes in water temperature.

The average duration of the feeding period on the east coast oyster beds may be deduced from records of sea temperature in the River Blackwater covering the five years 1926–30 given by Orton and Lewis (1931). It is apparent that on the average the water temperature exceeds 14° C. about mid-May and again

TABLE VIII. OYSTER SPAT DESTROYED PER DAY DURING 1941 BY 39 *UROSALPINX*, TOGETHER WITH THE MEANS OF THE DAILY READINGS OF MAXIMUM AND MINIMUM WATER TEMPERATURE

Date	Spat destroyed	Average water temperature ° C.
June		
17	1	17
18	0	18·5
19	0	18·5
20	0	18·5
21	0	16
22	0	No record
23	2	18
24	4	19
25	8	19·5
26	6	19
27	10	19·25
28	13	18·5
29	18	No record
30	9	19
July		
1	16	21
2	17	21·25
3	32	22
4	27	18·75
5	10	19
6	32	19·5
7	15	20
8	17	19
9	27	19
10	21	20
11	28	20·5
12	12	21
13	26	20·5
14	44	21·5
15	37	21·25
16	19	18·75
17	23	16·75
18	31	17
19	23	17
20	16	No record
21	31	16·5
22	28	15·75
23	29	15·25
24	37	17·75
25	45	19·25
26	32	18·75
27	37	No record
28	43	18·5
29	22	18
30	36	15·5
31	30	15·75
August		
1	36	16·5
2	31	18
3	40	18
4	31	17·5
5	48	15·5
6	27	13·25
7	24	13
8	16	14
9	19	15
10	22	15·75
11	23	16·5
12	30	16·75
13	29	15·5
14	24	15
15	26	14·5
16	21	13·75
17	19	14·5
18	21	15·75
19	24	16·75
20	22	16·75
21	16	16·25
22	11	15·75
23	17	16
24	16	15·25
25	17	15·25
26	27	16·75
27	13	16·5
28	15	15·25
29	12	16
30	11	14·75
31	15	15
Sept.		
1	28	16
2	22	16
3	21	16·25
4	24	17
5	22	17·25
6	31	18
7	22	17·5
8	19	17
9	29	17
10	12	16
11	26	15·5
12	16	14·5
13	15	14·25
14	18	No record
15	13	14·5
16	11	14
17	10	14·5
18	20	15·25
19	8	14·5
20	22	14
21	8	No record
22	23	14
23	12	14
24	10	14·25
25	10	14·25
26	13	15·25
27	9	15·5
28	12	16·25
29	9	16
30	8	15
Oct.		
1	2	13·5
2	0	13
3	3	13
4	3	12·5
5	1	No record
6	1	12·5
7	4	13·5
8	1	14
9	1	14·75
10	4	14·75
11	6	14·75
12	3	No record
13	2	13
14	2	11·5
15	2	11·5
16	1	10
17	1	11
18	1	10
19	2	No record
20	3	11·5
21	3	10·75
22	2	10

falls below this level early in October. The duration of the feeding period is therefore approximately 5 months. Assuming an average rate of destruction of 0·385 spat per day, one tingle feeding exclusively on such 1-year-old spat would destroy 59 spat. Valuing these spat at four to the penny, a conservative estimate, the potential saving for each tingle removed is 1*s*. 3*d*. per season.

According to Federighi (1931), *Urosalpinx* from Hampton Roads, Va., U.S.A., begins to feed when the temperature rises above 15° C. and ceases when the temperature falls below 10° C. Similar studies in Delaware Bay (Galtsoff *et al.* 1937) indicate that drilling ceases altogether at 9·5° C. Drilling had not ceased completely at Conway when the water temperature fell below 11° C. (Fig. 10), but activity was practically negligible at temperatures below 13° C. Galtsoff *et al.* (1937) obtained a good correlation between the number of oysters destroyed in baskets let down on to the oyster beds in New Jersey and the prevailing water temperature.

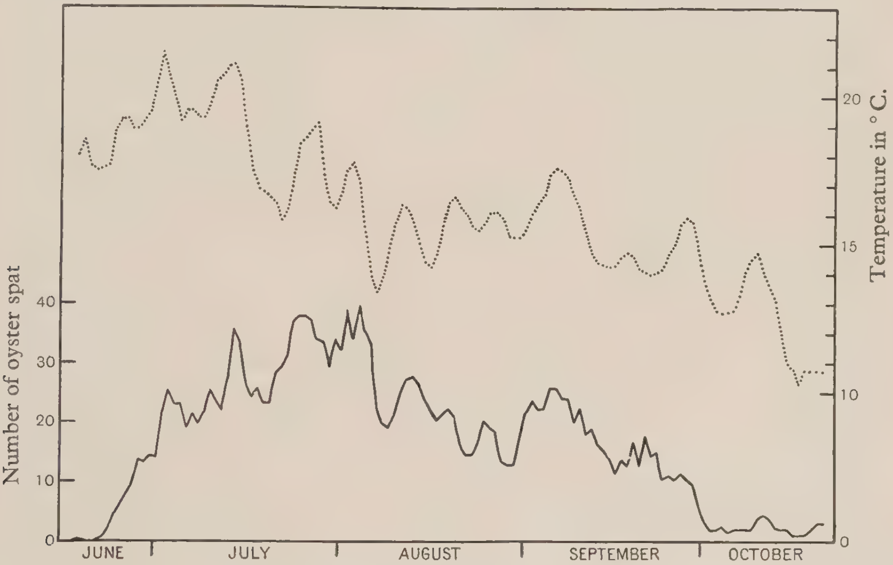


Fig. 10. Daily rate of destruction of oyster spat by 39 *Urosalpinx* during 1941 correlated with water temperature. Figures of both temperature and spat destroyed have been smoothed in threes.

The rate of destruction of spat and larger oysters as determined by American workers is considerably higher than that observed in the experimental cages at Conway. In the U.S.A. the ravages of *Urosalpinx* have been found to vary in different localities. In North Carolina waters a single *Urosalpinx* is stated (Galtsoff *et al.* 1937) to destroy 30–200 oysters in a season, depending on their size; the basis for this statement is not given. Certain other general statements are given, such as that of Nelson (1931), who states that in Delaware Bay *Urosalpinx* kills $1,000,000 worth of oysters annually, and that of Galtsoff *et al.* (1937) who say that there are many localities in Long Island Sound and in Chesapeake Bay where 60–70 % of the seed oysters are destroyed annually. These round figures are much on a par with the assertion by Essex cultivators that at least half the spat in the Blackwater is killed annually by tingles, a figure repeated by Orton (1937); there is a great lack of precise information on the subject. The only reasonably precise American determination of the rate of drilling is that of Galtsoff *et al.* (1937), who state that during a 9 weeks season in New Jersey waters each drill destroyed on the average 0·34 adult oysters per week. These workers also state that over 300 spat may be destroyed in a single season by each drill; the basis of this estimate is not given, but one suspects that the rate of destruction of spat has been determined for a short period in midsummer and multiplied by an appropriate factor depending on the relative length of the period of observation and the season of activity. If this is so, the estimate of total damage is clearly much too high, as it has been shown here that the maximum rate of drilling is only maintained for a short period in midsummer after the completion of spawning.

In order to determine the rate of destruction of newly settled spat by young tingles, 20 tingles which had been hatched under laboratory conditions were isolated with a plentiful supply of oyster spat which had settled the same season. The tingles averaged approximately 0·35 cm. in height, while the oyster spat varied in diameter from 3 to 7 mm. The experiment was conducted in a plunger jar with sea water of normal salinity, and the average water temperature during the period of the experiment was 20° C. A total of 162 spat was drilled and destroyed during a period of 140 hr. by the 20 young tingles, giving an average rate of 1·39 spat per day (24 hr.) per tingle. This experiment proves that freshly emerged tingles may cause extensive havoc amongst newly settled spat. At this stage the tingles are for practical purposes immune from capture. Young tingles feeding entirely on such small spat might destroy as many as 100 each during the period July to September inclusive.

Orton (1927), in the course of some preliminary experiments, states that the average time taken for *Urosalpinx* to drill through an oyster was 5·7 days; the size of these oysters is not given, nor the water temperature, but it is implied that the oysters were larger than brood size. It is not suggested, however, that *Urosalpinx* consumed oysters continually at this rate, but that the average rate of drilling in 15 cases was as stated above. His figure for brood oysters 1–2 in. long was 4·1 days (10 oysters), but eight brood oysters nearly bored through had been drilled for an average of 4·5 days before being abandoned. Orton gives a general estimate of the time taken to drill a brood oyster as 5–6 days. This is very different from the average rate of destruction of such oysters as determined at Conway, viz. 33 days; this does not mean that each drill takes 33 days to destroy an oyster, for drills do not continuously attack one oyster after another; considerable periods of inaction occur, so that the rate of drilling, as distinct from the rate of destruction, may be much the same as Orton observed.

The high rate of destruction of adult oysters observed in America (see above) is all the more remarkable when we recall that on the average no less than 33 days was needed to destroy one 2-year-old oyster under experimental conditions at Conway. We are forced to conclude either that *Urosalpinx* has changed its habits under the conditions on British beds or that the shell of *Ostrea edulis* presents a much more effective barrier to *Urosalpinx* than that of *Gryphaea virginica*. In this country the general belief among oystermen is that the American tingle is principally an enemy of the spat and early brood stages and that damage to marketable oysters is negligible. Careful observations on the beds and food-preference experiments at Conway confirm this view.

Food preference has been investigated by enclosing samples of tingles in floating cages with two or more sorts of food and noting daily the number of drills attacking each kind of food. Materials offered as food have been oysters of all ages from early settled spat to 3 years old, mussels, and barnacles. The drills used were obtained from the River Blackwater and were all over 2·5 cm. in height. Certain general conclusions emerge quite clearly from these experiments. Drills offered barnacles or spat oysters (1-year-old) divided their attention about equally between the two kinds of food. Drills offered spat oysters and mussels destroyed practically all the spat before attacking the mussels. When the spat were all finished the mussels were quickly destroyed, drilling occurring chiefly near the thin edge of the shell. Similarly barnacles were more attractive than mussels. Drills offered both spat and brood oysters (2 years old) destroyed practically all the spat before attacking the larger oysters. The latter were then attacked and slowly consumed. It was observed also that tingles showed a distinct preference for spat of thumb-nail size, not attacking very small spat until all the large spat had been destroyed.

We may therefore conclude that oyster spat and barnacles form the principal foods of *Urosalpinx* on the beds in the River Blackwater, but that larger oysters may also be attacked. Similar experiments made in America by Galtsoff *et al.* (1937) placed mussels ahead of seed oysters in the list of foods attractive to drills, barnacles being the most attractive of all. The lack of attractiveness of mussels to drills from the River Blackwater is probably due to the comparative scarcity of mussels in this river system. Orton (1929) has shown that *Ocenebra erinacea*, the British rough tingle, will attack most readily those food species which occur commonly in its normal habitat and that *Ocenebra* from areas where oysters do not occur will not readily attack oysters while other foods are available. It is possible therefore that *Urosalpinx* from, say, Whitstable, where mussels are abundant on certain parts of the ground, would exhibit a strong preference for this food. It is clear that in the River Blackwater *Urosalpinx* is primarily an enemy of the spat stage of the oyster.

Galtsoff *et al.* (1937) conclude from observations of feeding habits that drills are able to detect the presence of food and move towards it. As already noted, careful experiments made at Conway did not confirm this conclusion.

Economic Considerations

Since *Urosalpinx* has no free-swimming stage in its life history, and it is probable that migration is of no more than local significance, it is dependent for distribution upon transportation by human or other agencies to fresh areas.

There is therefore, theoretically, a good chance of localizing this pest and of controlling it in areas where it has already secured a foothold. The latter include, as already noted, the estuaries of the Blackwater, Colne and Crouch and the Kentish Flats off Whitstable. At present two methods of control are practised, in the first place all tingles seen are picked out of dredge hauls and taken ashore and, secondly, all spawn noted is collected and dried. Owing to the general depression in the oyster trade, brought about by the heavy mortality experienced as a result of the severe winters of 1939–40 and 1940–1, the amount of dredging done by the few boats that are now employed is insufficient to keep the pest in check, let alone eradicate it. The present slightly enhanced prices due to the general scarcity of oysters would normally result in rather more intensive working of the grounds; but this is hardly possible now on account of the shortage of suitable men and the operation of certain defence restrictions, e.g. the closing of parts of the oyster beds on the east coast, thus forming reservoirs in which tingles can multiply undisturbed. In normal dredging very few tingles less than three years old are captured, although these are most numerous on the grounds, simply because they are too small to be retained by the dredge bag, the rings of which are an inch across, and further, the close sifting of the dredge contents which is necessary to collect the smallest tingles is not in practice carried out. With the limited amount of labour employed and the scarcity of oysters on the grounds it is necessary to dredge and cull out as expeditiously as possible in order to secure enough oysters to pay for the day's working and consequently thorough sorting of the contents of the dredge bag is hardly possible. Further, many grounds on the east coast, partly as a result of infestation by *Crepidula* and partly as a result of the dying out of the *Zostera* which formerly clothed and consolidated the banks, have in recent years become rather muddy and it is necessary therefore to wash or 'dock' the dredge bag several times, by lifting it almost clear of the water and then dropping it back, in order to wash out mud and facilitate culling of the contents. Trials made by the writer when working on muddy ground at the mouth of the Blackwater showed that washing of the dredge to the extent necessary to free it from mud resulted in the loss of between 75 and 100% of the tingles. It has been recommended therefore that when dredging to clean fouled ground the usual docking of the dredge before shooting on deck should be omitted and any washing necessary carried out on deck with the aid of buckets. This is a slightly more tedious process but the catch of tingles is likely to be at least trebled.

Since *Urosalpinx* spawn hatches after an incubation period of 6–8 weeks, and the bulk of the spawn is deposited during May and June, hatching of spawn usually begins in early July. Consequently the period from the beginning of spawning, i.e. when the tingles first become active after the winter period of quiescence, until early July is the time during which maximum effort should be made to collect both tingles and spawn. After the beginning of July much of the spawn will have hatched. From other points of view the period from mid-April to early July is a favourable one for working the beds. It serves admirably for the collection and concentration on fresh ground of oysters destined for sale, both when the latter are to be sold as food in the autumn and when they are destined for replanting in other localities. This period is also the most favourable for cultivating the ground, picking out slipper limpets, and working the cultch over so as to render it suitable for picking up a spatfall in the period July to September.

The inefficiency of the dredge in collecting tingles below 3 years old is not so serious a matter as at first appears, for it is probable that the amount of spawn deposited by the small tingles is insignificant in relation to the production of larger tingles. Any reduction in the size of the rings forming the belly of the dredge will inevitably reduce the number of oysters caught per man on grounds carrying a lot of shell, due to the rapid clogging which will occur, and the increased numbers of tingles caught must be balanced against the reduction in the yield per dredge of oysters. It is, however, the practice in the Blackwater to use slightly smaller rings on certain grounds.

Although it is now generally recognized among oystermen on the east coast that tingles are a serious pest, much of the damage done passes unnoticed, for it is only when the clock of a brood or half-ware oyster is found with one of the valves drilled that visible evidence of the damage done is obtained. This will not occur very often, for serious damage to brood and larger oysters only occurs when spat is absent. In this country tingles are mainly an enemy of the spat, but a drilled spat is very rarely seen, for the drilled valve, usually by the nature of the situation of the spat the flat valve, generally becomes detached and broken up almost immediately after the spat gapes. Practically the only evidence of the activity of tingles is therefore the failure of the spatfall to show up the following season. Similarly it is practically impossible to detect the damage done by freshly emerged tingles among the tiny spat of the season; yet it has been shown that these small tingles may destroy these spat at an astonishing rate. Since no method is known or is likely to be devised for trapping these very small tingles the best way of controlling their activities is to prevent them emerging by collecting the spawn before the beginning of July. It will be noted that the time of emergence of tingles coincides with the beginning of the normal spat settlement period.

Until a few years ago a bonus was paid to dredgermen on the basis of the number of tingles and clumps of spawn brought in. This has now been discontinued, partly because of the difficulty of checking the actual numbers and partly because the extra payment for tingles was found to result in what was considered to be undue attention being paid to this aspect of the work on the beds to the detriment of the numbers of oysters taken per man per day. It would probably pay to re-introduce this system where the main task involved is the cleaning and cultivation of ground rather than the collection of oysters. The payment involved was small, e.g. 1*s*. per 1000 on the River Roach. The amount paid out during the year 1936–7 was £9. 5*s*. 0*d*., representing a total of 185,000 tingles and spawn. The main operation on which the company was engaged at the time was the clearing of the ground prior to laying down a large consignment of oysters from Brittany, and the ground was being intensively worked by a large number of boats. The following season payments fell to £3. 11*s*. 6*d*., representing only 71,500 tingles, showing that tingles may be greatly reduced by dredging alone when intensive working of the ground is possible.

As mentioned earlier a conservative estimate of the value of the damage due to a single tingle feeding entirely on oyster spat is 1*s*. 3*d*. per season, while should the tingle prove to be a female and be removed before spawning the potential saving is much greater. It is possible therefore to justify on financial grounds any reasonable sum spent on tingle control, for a man engaged solely upon this work and being paid £3 per week needs to catch only eight per day to pay his way on the basis of the above estimate. When it is realized that on some beaches in the Blackwater river system a man can collect 500 tingles in a few hours from around low-water mark, then there can be no doubt that the detachment of men from normal dredging operations for the work of hand-picking on such beaches is well justified.

In the U.S.A. special tingle traps and dredges have been the subject of large-scale experiment with very encouraging results (see Galtsoff *et al*. 1937). Some of the simpler methods of control advocated in America are already incorporated in normal practice over here. These methods all aim at the removal of drills from oysters after dredging and include screening, forking, hand-picking, etc. This is of course already carried out on British beds; no oysterman in this country knowingly throws a tingle back once it has come on board.

Another method of control strongly advocated by Galtsoff *et al*. (1937) is the use of special dredges fitted with a perforated pan to retain tingles but allow oysters to escape. Although these special dredges have never been tried in England the consensus of opinion is that they would clog up immediately on the clay grounds of the Essex rivers. They might work well on the Kentish Flats, but tingles do not yet present a severe problem there and present methods of control are fairly adequate.

Several different kinds of drill traps have been tried in America, with apparently considerable success. One type of trap takes advantage of the habit of the drills of congregating on objects raised above the bottom for the purpose of depositing egg capsules. Small concrete pillars, wire bags filled with shells, bunches of large shells or tin cans wired together, all are stated to attract spawning drills. They are laid out attached to lines and buoys and fished every few days, being taken up and moved to fresh areas as the catch diminishes. The use of such traps is to be strongly recommended in this country for the habits of the spawning tingles are the same; further, half-sections of large drain-pipes laid down on the beach at low water of spring tides in Thornfleet have attracted large numbers of drills and show that trapping can be carried out with success.

SUMMARY

The American whelk tingle, *Urosalpinx cinerea* (Say), a gastropod enemy of oysters, has been introduced to Britain with American oysters and is now established in the rivers Blackwater, Colne, Crouch and Roach in Essex and on the Kentish Flats off Whitstable. Evidence available concerning the migratory powers of *Urosalpinx* is conflicting, but it is probable that migration is of local significance only in the spread of this pest. To infest new areas tingles or their spawn must be transported by human or other agency, for *Urosalpinx* has no free-swimming larval stage, the eggs being deposited in capsules from which fully formed young tingles emerge.

Females of *Urosalpinx* grow more quickly than males and reach a larger size. In males the annual increase in shell height never exceeds 0·2 cm. after the first 3 years, and is generally rather less. In females an annual increment of 0·2 cm., or slightly more, is general up to the age of about 7 years, after which it gradually falls. Males and females of the same age differ in height by about 0·25 cm. Growth marks are frequently to be seen on the shell and are of value in assessing growth rates. In both sexes a maximum age of 13–14 years may be reached. *Urosalpinx* reaches a much greater average size in Britain than in its natural habitat on the Atlantic coast of the U.S.A.

Females may deposit some spawn during the first 2 years of life, but the amount is insignificant in relation to the production of older tingles. Spawning begins when the water temperature in its seasonal rise reaches 12° C. Adult females may deposit an average of 25 egg capsules at a single laying, but it is possible that further capsules are deposited later in the season. The bulk of the spawn is deposited in May and June on British beds, but a few freshly laid capsules may sometimes be found in August and September. The average period of incubation is about eight weeks. Young tingles usually begin to emerge early in July. The average number of young emerging from each capsule is 11·74. *Urosalpinx* appears to be more prolific on British oyster beds than in America.

Urosalpinx from the River Blackwater exhibits a preference for oyster spat and barnacles over other foods. Oysters larger than spat are not seriously damaged when spat are available. Oyster spat and barnacles are about equally attractive. Tingles confined with oyster spat destroyed them at an average rate of 0·385 per tingle per day over the whole feeding period, but, for a period during July, spat were destroyed at the rate of 0·9 per tingle per day. Two-year-old oysters were destroyed at the rate of 0·033 per tingle per day. The rate of destruction is correlated with water temperature. Drilling may begin when the water temperature exceeds 11–12° C., but little feeding occurs while the tingles are breeding in May and June. Feeding usually ceases early in October and tingles remain quiescent throughout the winter until the water temperature again reaches 12° C., when spawning begins. Freshly emerged

tingles may cause much damage among oyster spat which has recently settled.

Control of this pest is discussed in the light of the information available concerning its biology and distribution.

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THE DISTRIBUTION OF *ARACHNACTIS ALBIDA* M. SARS IN THE CELTIC SEA

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(Text-fig. 1)

The present paper is an account of the distribution of *Arachnactis albida* (Fam. Arachnanthidae, Carlgren, 1924) in the area south of Ireland and in the western approach of the English Channel during 1937–9. This larval Cerianthid, the adult of which is at present unknown, is a North Atlantic planktonic species. It has been collected by numerous scientific expeditions, the records of which indicate a centre of distribution between the Faroes and the Hebrides with a range extending along the Atlantic seaboard of north-western Europe from the Barents Sea to south-west of Ireland, and as far west as long. 20° W. (Carlgren, 1906, 1924). It has been taken in the northern North Sea frequently, but in the southern North Sea the only record is of a solitary specimen found off the Norfolk coast (lat. 50° 02′ N., long. 1° 40′ E.) by Leloup (1929, 1931), who attributes its occurrence there to a south-going coastal current. There is no valid record of the species in the English Channel or the Irish Sea, but off the west coast of Ireland specimens have been taken from many points, especially towards the south-west in Valentia Harbour (Browne, 1900) and off Tearaght Light (Bourne, 1920). This is the southernmost point (lat. 51° 27′ N., long. 11° 51′ W.) at which it has been recorded by previous workers. According to Carlgren's investigations (1906), several of the records of *A. albida* in the International Bulletins refer to other species (Kramp, 1913), and the records in the *Bulletin Planktonique* are not reliable as *A. albida* has been confused with *Synarachnactis bournei*[1] (Ostenfeld, 1931, p. 632). The records show that the species occurs mainly in open oceanic seas and not in the relatively more enclosed waters of the English Channel, Irish Sea and the southern North Sea (excepting Leloup's single record). Bourne, who investigated the specimens collected off south-west Ireland, suggested a subcentre of distribution in that area (near lat. 51° N.), but he emphasized that 'the possibility must not be overlooked that the larvae and adult or quasi-adult forms taken off the south-west coast of Ireland belong to a species or sub-species distinct from *A. albida*' (Bourne, 1920, p. 45). His statement is based on evidence of 'differences in size, colour(?), distribution and minute anatomy' between the specimens from the south-west coast of Ireland and examples from the Faroes. This problem is discussed in the present paper.

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[1] This is the species of *Arachnactis* which occurs at Plymouth in spring and summer (vide *Plymouth Marine Fauna*, 1931, p. 87). *A. albida* has not been found at Plymouth. Bourne's record (1890, p. 321), cited by many authors, actually refers to *Synarachnactis bournei* which is the larva of *Cerianthus lloydi* (vide Bourne, 1920, p. 29). The statement in *Journ. Mar. Biol. Assoc.*, Vol. VII, p. 203, 1904, concerning the occurrence of *Arachnactis albida* in spring at Plymouth is a mistake, repeated by Pax (1934), who has overlooked the correction in the 1931 edition of the Plymouth Marine Fauna.

The specimens of *Arachnactis* taken during 1937–9 in the Celtic Sea[1] can be referred with certainty to the species *A. albida*. A careful analysis of distribution in the area was made not only in view of the new locality but also because there is no accurate information concerning its distribution towards the south. The present data extend the range of the species as far south as lat. 48° N. off the mouth of the English Channel and indicate the existence of a localized subcentre of distribution to the south of Ireland in the neighbourhood of lat. 50° N., long. 10° W. Its occurrence to the south and east of this new centre can probably be correlated with water movements.

The material for this study was obtained in 1937–9 during a series of spring and summer cruises undertaken in the course of the Plymouth mackerel investigations and covering the area of the south-western continental shelf. The plankton samples were collected by means of half-hour oblique hauls with the 2 m. stramin ring-trawl. A thorough examination was made of the material obtained in June 1939, for which year hydrographical data were also available. The larvae were picked out from each haul and their total numbers counted except at those stations where they occurred in swarms; in the latter the total was estimated by subsampling. The plankton collections of the other cruises were then examined in order to determine whether the centre of distri-

TABLE I. *Records of* Arachnactis albida *from the Celtic Sea, arranged according to months*

Cruise	Station no.	No. of *Arachnactis*	Cruise	Station no.	No. of *Arachnactis*
March 1939	5	0	June 1939[2]	2	1
	18	0		3	1
				6	44
April 1938	19	0		7	3
	20	0		8	4
	21	0		9	1
				10	1050
April 1939	23	0		11	600
	24	1		12	1
				14	2
May–June 1937	5	0		15	47
	8	0		18	2
	10	0		19	2
	15	0		27	5
June 1938	6	0	July 1937	5	0
	13	1		8	102
	18	3000		10	0
	28	0			
	30	0			

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[1] 'Le terme de "mer Celtique" fut employé pour la première fois par E. W. L. Holt, pour désigner l'étendue marine qui recouvre le plateau continental au sud de l'Irlande et à l'Ouest de l'entrée de la Manche' (Le Danois, 1938). Though we have not been able to find it in any of Holt's published papers, this term is used here owing to its great convenience.

[2] See Fig. 1 for the fourteen stations at which no larvae were obtained in this cruise.

bution was similarly located in each year and also to ascertain the time of year at which the larvae begin to appear in the plankton. Owing to the large number of samples this examination was not made station by station: it was limited to certain hauls from the areas in which *A. albida* was abundant in June 1939 and to certain other hauls from areas barren of larvae in June 1939. This was considered fully sufficient, since all the samples had previously been examined minutely in the course of the mackerel work and the presence of *Arachnactis* larvae had been noted at the time. It is therefore unlikely that their occurrence in any numbers escaped notice. These results are summarized in Table I.

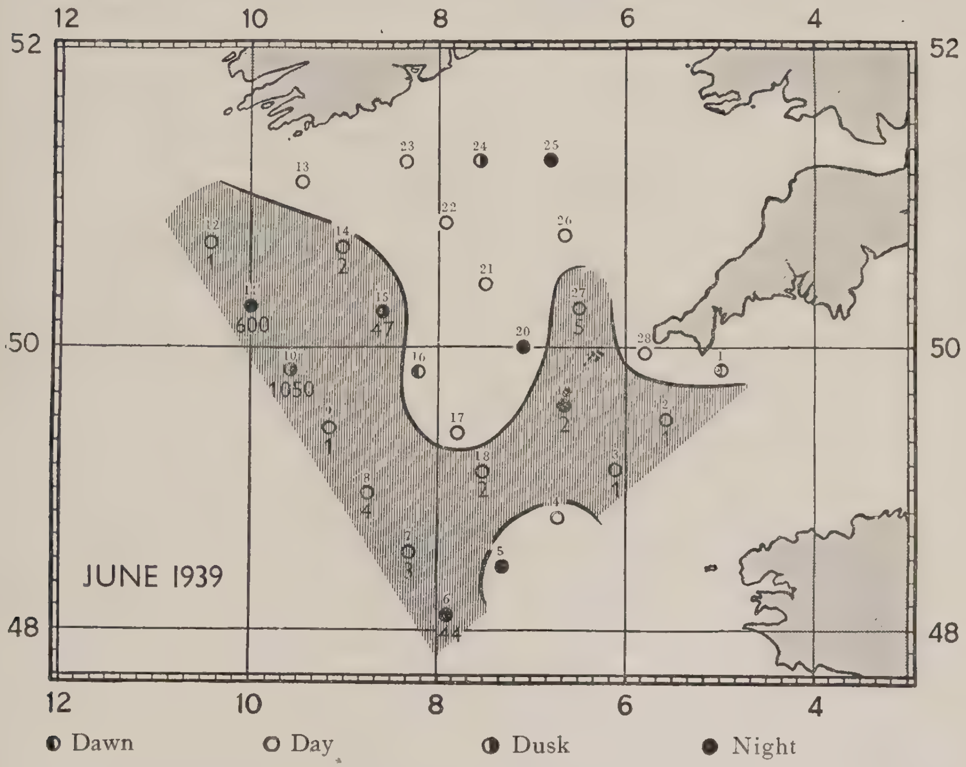

Fig. 1. Distribution of *Arachnactis albida* in the Celtic Sea in June 1939. Shaded area shows regions where larvae were obtained. Circles indicate station positions (differently shaded according to time of haul) with the station numbers above. The number of larvae obtained (if any) is shown below each circle.

The numbers of *A. albida* obtained in June 1939 are shown in this table and in Fig. 1. The species was widely distributed over the western, southern and eastern margins of the area investigated—almost completely surrounding a region barren of larvae which extended southwards from the St George's and Bristol Channels (Sts. 13, 16, 17 and 20–26). The largest catches were taken at Sts. 10 and 11, where 1050 and 600 specimens respectively were recorded. Elsewhere only small numbers occurred, but 44 larvae were taken at St. 6, which is noteworthy since it is the southernmost record of the species. The large hauls at Sts. 10 and 11 suggest the existence of a distribution centre in this region. Since, however, both day and night hauls were taken during the cruise, this indication cannot be established without first determining whether or not the observed horizontal distribution was affected by diurnal vertical migration of the larvae. This is especially important since Sts. 10 and 11 were worked at dusk and just before dawn. Sts. 6 and 15, where the next largest catches were made (44 and 47 specimens), were worked just before dawn and at midnight respectively. Unfortunately there is little information relating to the diurnal behaviour of *A. albida*, and the question can therefore only be further investigated by a critical examination of the data available in the present records.

In June 1939 *A. albida* was taken at fourteen stations. A total of twenty larvae were taken at nine day stations in contrast to over 1700 from one dusk and four night stations (Sts. 6, 10, 11, 15 and 19). The four largest catches of *A. albida* were all from night stations. This suggests that the large numbers taken in night hauls were probably due to migration of the larvae into the upper layers of water during darkness. Apart, however, from any temporary night-time increase in numbers of larvae near the surface, the existence of a distribution centre in June 1939 is indicated by the marked difference between the large total of 1650 larvae from Sts. 10 and 11 and the much smaller total of only 91 from the two next largest night catches at Sts. 6 and 15. Vanhoffen (1895) has noted the occurrence of *A. albida* in swarms at the surface in daytime. A daylight haul in July 1937 contained 102 specimens, and it is significant that about 3000 *A. albida* were taken in a daylight haul in June 1938 at a point less than 5 miles distant from St. 10 of June 1939. This recurrence in two consecutive years (1938 and 1939) of dense *Arachnactis* patches in the same locality and at the same time of year affords strong evidence of the existence of a breeding centre in the neighbourhood of lat. 50° N., long 10° W.

All the larvae taken in June 1939 were at fairly early stages of development. The youngest example had only five marginal and two oral tentacles, a stage very similar to that illustrated by Bourne (1920), which is the youngest larva recorded. The oldest individuals had eleven marginal and six oral tentacles. Of the 1050 specimens from St. 10, a critical examination of two subsamples showed that about 10 % were early stages in which the directive tentacle had not appeared, about 2 % possessed more than nine marginal tentacles, and the bulk of the material belonged to intervening stages in which the directive was recently formed and distinctly smaller than the other marginal tentacles. Larvae from Sts. 11 and 15 showed about the same percentages. Larvae from Sts. 6 and 27 were mostly earlier stages with less than eight marginal tentacles, and the single specimens obtained at Sts. 2, 3 and 9 were damaged, though certainly identifiable as *A. albida*. No *A. albida* were taken on the June 1937 cruise. This may in some measure have been due to the fact that the area investigated did not extend farther west than long. 8° 30′ W. But in July 1937, 102 specimens were taken at a point about 50 miles south-east of the June 1939 centre. Most of these larvae were older stages than those found in June 1939; they had more than six oral tentacles, and the marginals had begun to drop off prior to the settlement of the larvae.

On the whole the records of 1937–9 show considerable agreement in regard to the locality of the dense concentrations of *A. albida*—a fact which points to the existence of a localized centre of distribution in the area investigated. It is also evident that in this area early June is the period of abundance, whereas in the Faroe-Hebrides region, July and August are the months during which the species has been most commonly taken. The single larva taken in April 1939 (about 20 miles west of St. 10 of June 1939) is the earliest record from the Celtic Sea. It would appear that in the south adults of *A. albida* begin to breed earlier than in northern latitudes, an observation which is in accord with the breeding behaviour of many other species.

It is probable that the centre of distribution off the south-west of Ireland is not as localized as is indicated by either Bourne's or the present records. Further work in the area may reveal that it covers a very much wider region embracing both these sets of records; or that the position of the centre of distribution shifts with the changes in flow of the Atlantic Stream (Harvey, 1930) or with changes of other controlling factors not yet known.

The *Arachnactis* obtained in the Celtic Sea during 1937–9 all belong to the species *A. albida*. The external appearance of the larvae, the arrangement of tentacles, colouring and disposition of the mesenteries as revealed by dissection of older individuals agree completely with the descriptions of *A. albida* given by Carlgren, Vanhoffen and others. The earlier stages show the characteristic brown coloration at the tips of the tentacles and on the throat. Examination of *A. albida* from the Faroe Channel[1] and comparison of them with the Celtic Sea specimens showed complete agreement in regard to external features.

It has also been possible to examine and compare a large series of larvae collected from the west coast of Ireland[2] with the Celtic Sea material. Some of these were taken to the north in the neighbourhood of Cleggan Head, Co. Galway, others to the south near Tearaght Light, the original locality of Bourne's specimens. From this comparison there can be no doubt that the larvae from the west coast of Ireland are identical with those from the Celtic Sea. It is therefore necessary to re-examine Bourne's suggestion of a distinct Irish species or subspecies which, it will be recalled, was based on differences in distribution, size, colour and certain details of micro-anatomy. The Celtic Sea records, together with those from the west coast of Ireland and the numerous records from the Faroe-Hebrides area clearly indicate that *A. albida* has a continuous distribution along the western seaboard of the British Isles. The specimens from the neighbourhood of Cleggan Head do not appear to have come to Bourne's notice. This may possibly account for Bourne's statement that the 'northern form' has a distribution entirely separate from that of the 'Irish form'. With regard to colour, Bourne stressed the absence from his specimens of the brown coloration of the throat region which is typical of *A. albida.* He examined the specimens several years after their collection and noted that the throat coloration might have been lost since capture. In this connexion, it may be mentioned that the larvae obtained in June 1939 clearly showed the characteristic brown coloration at the tips of the marginal tentacles and in the throat, whereas this had already begun to fade, especially from the throat, in the larvae taken during the earlier cruises of 1937 and 1938. All the Celtic Sea larvae were examined in 1941. This possibility of fading during storage considerably reduces the importance which Bourne attached to the colour differences between his specimens and typical *A. albida.* The third difference noted by Bourne is that of size. His specimens from the south-west coast of Ireland were smaller than larvae from the Faroe Channel. On the whole, the Celtic Sea larvae were also smaller when compared with the measurements given by Vanhoffen and others. Considerable size variation among larvae of the same developmental stages was noticeable in the Celtic Sea material and also in the specimens from the Faroe Channel. This slight size difference alone cannot be considered sufficiently important a criterion upon which to base a specific or subspecific difference. Finally, the minor anatomical differences observed by Bourne are not of value from a systematic standpoint, and the morphology of the typical *A. albida* as described by Carlgren, Vanhoffen and others, is essentially the same as that of the Irish specimens studied by Bourne. It would thus seem that there is no justification for the separation of the *Arachnactis* from the south-west of Ireland into a species or subspecies distinct from the *A. albida* of the Celtic Sea and the Faroe-Hebrides region.

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[1] These specimens were supplied through the courtesy of Capt. A. K. Totton of the British Museum.

[2] These specimens were collected during May–September 1890–1905 by the Irish Fisheries Department, and were kindly handed over for examination by Mr W. J. Rees. They were formerly part of the late Mr E. T. Browne's collection.

The distribution of *A. albida* in the Celtic Sea in June 1939 seems to show some correlation with the salinities of the surface layers (for chart of salinities at 5 m., vide Mare, 1940, p. 477). The larvae were present where salinities were greater than 35·2 ‰ but were entirely absent from the central tongue of lower salinity water (< 35·2 ‰), extending southwards from the St George's and Bristol Channels. The centre of distribution lay in high salinity water (> 35·4 ‰) which was present at all depths along the edge of the continental shelf. Salinities at depths less than 25 m. probably varied little, but at about 25 m. there were indications from both salinity and temperature values of a sharp discontinuity. In the deeper water of 50 m. there was less correlation between salinity and the occurrence of larvae.

Matthews (1914), Harvey (1929, 1930) and others have given a general description of the cyclonic circulation of water in the Celtic Sea and its bearing on the distribution of many species is reviewed by Russell (1939). The salinities observed in June 1939 support this picture of circulation, and the correlation between surface salinities and the occurrence of larvae indicates that water movements in the area may have a marked influence on the dispersal and distribution of *Arachnactis*. The small numbers of larvae obtained outside their centre of distribution (Sts. 2, 3 and 19 off the Channel mouth and St. 27 north of the Scillies) were probably carried there by the cyclonic current.

It is of interest to note that Mare (1940), working on the phytoplankton of the Celtic Sea, found some evidence of mixing of Atlantic and Channel water close to the Scillies in the occurrence in April 1939 of both oceanic and neritic species of diatoms. In June 1939 a surface patch of high salinity water ($> 35 \cdot 5$ ‰) at St. 27 again indicated the presence of water of mixed origin in the Land's End-Scillies channel, but Mare states that no phytoplankton species was especially associated with it. Some few *A. albida* were, however, taken at this station; they were all early epiplanktonic stages which may well have been carried there by water movements.

It is probable that the adult of *A. albida* is a benthic species (Carlgren, 1931). In the Celtic Sea adult examples, liberating larvae in May and June, may be expected to occur near the edge of the continental shelf in the area round lat. 50° N., long. 10° W.

It remains to be seen from future work if the correlation between water movements and the occurrence of *A. albida*, here indicated, is reliable, for the species may then prove to be a useful summer indicator of oceanic water in the Celtic Sea.

The authors wish to acknowledge their indebtedness to the Director and Staff of the Marine Biological Laboratory, Plymouth, where the work was carried out. P. G. Corbin also wishes to make acknowledgements to the Trustees of the Leverhulme Research Fellowships for a research grant which enabled him to carry out his share of the work.

SUMMARY

The distribution of *Arachnactis albida* in the Celtic Sea in 1937–9 is described. The species appears to have a definite centre of distribution in the neighbourhood of lat. 50° N., long. 10° W. The larvae have been taken as far south as lat. 48°, and eastwards up to the mouth of the English Channel, but they are absent from the tongue of low salinity water continuous with the St George's and Bristol Channels. Their distribution in the remaining area can be correlated with the cyclonic circulation.

The larvae obtained from the Celtic Sea are specifically identical with those found off the west coast of Ireland and with the typical *A. albida* of the Faroe-Hebrides region.

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A STUDY OF A MARINE BENTHIC COMMUNITY WITH SPECIAL REFERENCE TO THE MICRO-ORGANISMS

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(Text-figs. 1–3)

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INTRODUCTION

The object of these investigations is to make some contribution to the understanding of that part of the marine food cycle which takes place on the sea bottom. A marine mud sediment, off Rame Head near Plymouth, under relatively open sea conditions was chosen for this study. A preliminary account[1] is first given of the quantities and types of micro-organisms found in the surface layers of the deposit. In such a place organic matter is continually settling, and under aerobic conditions a rich and varied population of micro-organisms is present. An attempt has been made to treat these micro-organisms as a community. Special attention was paid to the surface layer because it is an important feeding zone for detritus-eating members of the macrofauna, and a subsidiary problem was the question of the relative importance of detritus and micro-organisms as the actual food supply of the macrofauna. Under the term *detritus* is included all material of living origin at any stage of disintegration and decomposition, from recently dead plant or animal or part thereof to the finest particle. The final stages of some of the products of decomposition, being colloidal or true solutions, are not included under the term; they are likely to be of food value to bacteria and minute saprophytes.

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[1] Owing to the war further samples could not be obtained.

A brief census was also made of most of the larger fauna. A census of organisms over the whole range of individual size is a necessary preliminary to the understanding of quantitative food relationships throughout the community of micro-organisms and larger animals. It is also necessary for assessing the relative importance of the various groups in the utilization of deposited phytoplankton and detritus and the extent to which loss of organic matter from circulation is prevented. Fig. 1 shows the main phases of the complex marine food cycle. The parts with which this paper is specially concerned are indicated by heavy type. The part which takes place in the overlying water masses has been described for the Plymouth area by Harvey, Cooper, Lebour & Russell (1935), and data on the phytoplankton crop and its consumption during the period when these bottom investigations were in progress is given in Mare (1940). The turn-over in the water (shown at the top of Fig. 1) is probably rapid and considerable (Gardiner, 1937, Harvey, 1940, 1942), but in shallow water events on the bottom merit some consideration although their relative importance in the general cycle is as yet unknown.

The productivity of the sea bottom was the subject of important contributions by Petersen and Boysen Jensen (1911, 1914, 1918, 1919) and Blegvad (1914). These workers were concerned with the macrofauna, its sources of food supply and its utilization as fish food. There have since been numerous papers dealing with the macrofauna and some with other aspects of the subject. An investigation of the food of the macrofauna made in the Plymouth area by Hunt (1925) showed clearly the need for quantitative work on the smaller organisms of the benthic fauna and flora. Several workers have studied copepods, nematodes, foraminifera, etc., in submarine deposits and also the bacteria; but except for qualitative work on the protozoa by Remane (1933) and Lackey (1936) no ecological study has previously been made of the protozoa and algae of the marine benthos.

On the bottom is a fauna of detritus eaters and predators which may be separated for convenience according to size or weight into three groups. Associated with these differences is another factor, the generation time, which, though by no means easy to determine, varies enormously between the groups and is important in considering the role of the various organisms in the community. A new terminology is needed, and these groups are here designated the *macrobenthos*, *meiobenthos*[1] and *microbenthos*. The macrobenthos is equivalent to the macrofauna of the bottom, and would also include large attached

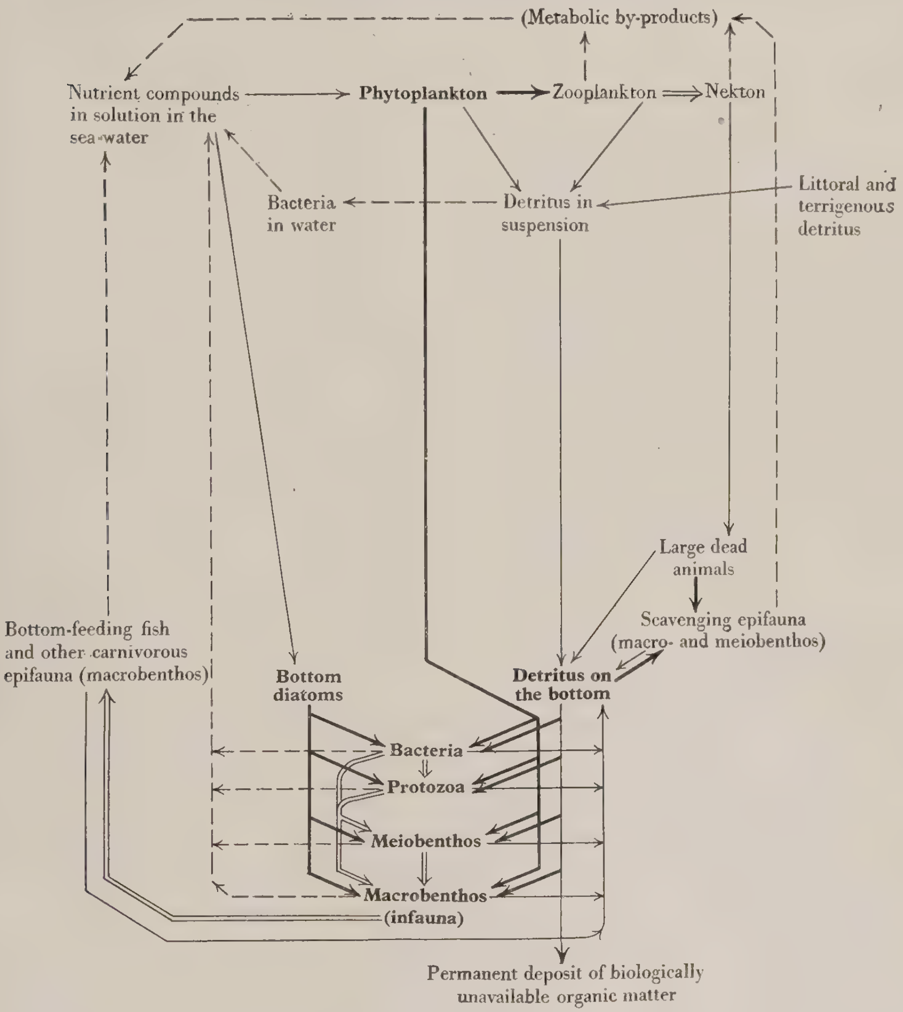
(Metabolic by-products)
Nutrient compounds in solution in the sea-water
Phytoplankton
Zooplankton
Nekton
Bacteria in water
Detritus in suspension
Littoral and terrigenous detritus
Large dead animals
Scavenging epifauna (macro- and meiobenthos)
Bottom-feeding fish and other carnivorous epifauna (macrobenthos)
Bottom diatoms
Detritus on the bottom
Bacteria
Protozoa
Meiobenthos
Macrobenthos (infauna)
Permanent deposit of biologically unavailable organic matter

Fig. 1. Diagram of the main phases of the marine food cycle. Above are stages which take place in the water and below are those which take place on the bottom surface and in the deposit. Heavy type indicates the material with which this paper is concerned. Plant and detritus consumption, ⟶; predatory relationships ⟹; metabolic changes, — — →; other changes, ⟶.

algae in habitats where they occur. The meiobenthos here comprises the fauna of intermediate size, such as small crustacea (copepods, cumaceans, etc.) small polychaetes and lamellibranchs, nematodes and foraminifera. The microbenthos comprises all the small organisms—protozoa (excluding foraminifera, i.e. ciliates, amoebae and flagellates), bottom diatoms and bacteria. The word micro-organisms here denotes all the members of the microbenthos. The fauna of intermediate size has been called the microfauna by Krogh & Spärck (1936) and by Rees (1940), and Remane used the term to include small metazoa and some protozoa. In a freshwater deposit Rawson (1930) employed a similar usage, including small metazoa and such protozoa as were found by direct searching under the term microfauna; some small metazoa appeared also in the lists of the macrofauna and he did not consider algae and bacteria. This use of the term seems rather unsatisfactory and inadequate for the present study. Bacteria are frequently called the micro-organisms of the sea bottom, but the omission of protozoa and diatoms has been largely on account of lack of knowledge of these groups. The exact limits of the macro-, meio- and microbenthos will probably vary according to the habitat under consideration and the methods which have to be employed for collection. The two larger groups can be separated according to size, depending on the mesh of the sieves employed for their extraction from the deposit. The groups can also be separated on the basis of weight, the weights of the microbenthos being deduced from volumes. The difference in generation time provides a further reason for separating the foraminifera with the small metazoa from the microbenthos. For a more exact definition of the limits of the groups in the habitat here described see Table VIII, p. 545.

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[1] Greek *meion*, smaller.

This work was carried out at the Plymouth Laboratory while I was holding a Newnham College Research Studentship and a grant from the Department of Scientific and Industrial Research. I wish to thank the Director of the Laboratory for the numerous facilities given me, and to thank the staff, particularly Mr G. A. Steven, and visiting research workers for many helpful discussions during the course of the work, and also Prof. J. Gray, Dr M. Rosenberg and Dr J. E. Smith for helpful criticism of the typescript.

THE HABITAT

The station at which regular samples were taken is about one mile west-south-west of Rame Head, to the west of Plymouth Sound, at a depth of about 45 m. The conditions are those of a relatively shallow open coast, where the bottom is sometimes considerably disturbed by storms. The habitat thus differs from any previously described in detail by workers on the meio- and microbenthos, such as bottom stations in the Clyde worked by Moore (1931) and Lloyd (1931) and from the sheltered Danish waters.

Samples were collected by means of a weighted metal tube into which fitted a glass lining tube, with a plate valve at the upper end. This core sampling apparatus was a modification of that designed by Moore & Neill (1930). It brought up cores 5–15 cm. long with the surface layers practically undisturbed.

The deposit at the Rame station was a sandy mud, fawn to grey in colour. The amount of coarse material (5–0·5 mm. in diameter) was very variable in different samples, but the fraction of greatest weight was always made up of particles 0·25–0·1 mm. in diameter, and very fine silt (from 40μ to less than 1μ in diameter) contributed about 25 % of the dry weight and was, of course, important in determining the texture. There was no clear demarcation into zones within the depth sampled.

All water was rapidly expelled by heating at 110° C. The surface layers were more fluid than the rest, which retained its form when removed from the tube. In the surface $\frac{1}{2}$ cm. layer the water content was about 57 % of the wet weight and 30–33 % at a depth of 5 cm. It was noticed that in a set of samples collected after rough weather the loose texture of the surface mud extended appreciably farther down the cores. The volume of interstitial space was found to be approximately the same as that occupied by the particles. The fine silt lying between the larger particles could easily be brushed aside by motile members of the microbenthos, for which the microscopic topography is an important factor. Active holotrichous ciliates about $28 \times 10\mu$ in size were common, and hypotrichous forms, up to 50μ long, have been seen. Nematodes and polychaetes would burrow between the larger particles and ingest some of the finer ones. Harpactacid copepods seemed to be restricted almost entirely to the less compact surface layer. This restriction was probably also correlated with oxygen supply.

The total organic carbon content was about 1 % of the dry weight of the deposit, as determined by a wet dichromate oxidation method, slightly modified from Anderson (1939). Coal was frequently detected in samples of the deposit from this and other stations near the port of Plymouth, but fortunately it was scarcely oxidized by the method here used. The presence of coal as a contaminant in bottom samples has received little mention previously (see Trask, 1932). By no means all the organic detritus on the sea bottom is of equal use to the fauna or to the bacteria. Waksman & Hotchkiss (1938) and Anderson (1939) have found that the biologically utilizable fraction, as indicated by the oxygen consumption of micro-organisms oxidizing it and feeding on it in culture, represents only a small part (about one-sixth to one-tenth) of the organic matter found by the dichromate method.

Only on prolonged standing in the laboratory did the cores develop irregular black patches due to the presence of sulphide; it was therefore thought probable that the habitat normally contained some free oxygen in the surface layers.

Microscopic investigation of the deposit gave a further picture of the environment of the micro-organisms. Mud was frequently compacted into faecal pellets by the activities of the small polychaetes and lamellibranchs. No deposition of recognizable green faecal pellets of planktonic copepods, such as was found by Moore (1931) in the Clyde, was detected, although such pellets were common in the water. Large particles in the deposit consisted of siliceous and calcareous skeletal fragments often with pores or cavities. While these still contained organic remains they would probably cause aggregations of bacteria and protozoa therein (thus similar difficulties in dispersing the micro-organisms prior to counting arose with this material as with land soil). The following list was typical of the objects encountered, and it gives an indication of the sources of supply to the region.

	Origin
Moss shoots and leaves (rare fragments) Wood with medullary rays (rare fragments) Oak leaf (rare fragments)	Terrigenous
Laminaria stipe (rare fragments) Red and brown filamentous algae (fragments)	Littoral benthic
Hydroid spp. (fragments) Polyzoan spp. (fragments) Sponge spicules Echinoderm spicules	Benthic (including intertidal)
Polychaete chaetae Crustacean setae Crustacean limbs, empty Diatom frustules	Planktonic or benthic (some indigenous)
Ostracod valves Foraminifera, mostly dead	Benthic (some indigenous)
Living ciliates and flagellates *very* rarely seen Living copepods, nematodes, etc.	Indigenous fauna
Living diatoms:	
Gyrosigma sp., *Navicula* sp.	Indigenous benthic or tychopelagic
Paralia sulcata	Tychopelagic
Coscinodiscus sp., *Biddulphia regia* *Thalassiothrix Nitzschioides*, *Bacillaria paradoxa*	Planktonic
Faecal pellets, ovoid, consisting of mud	Products of the smaller macrobenthos

Note. The *littoral zone* is here used to include the intertidal region and down to the limits of attached vegetation. The Rame station is regarded as being in the *sublittoral zone*, in which it now seems very probable that pennate diatoms are present and active (see pp. 528 and 535). The term *tychopelagic* is applied to organisms which are associated with the bottom and may be washed up into the plankton. Diatom nomenclature is that used by Lebour (1930).

The nature and quantity of the organic fragments occurring on the bottom is dependent on the depth, the distance from land and on the local conditions of settlement. A few samples taken with the Hunt vacuum grab (Hunt, 1926) from coarse deposits farther seawards and taken from intertidal stations at Wembury and Laira illustrated the influence of these factors and also showed an interesting distribution of planktonic and bottom diatoms (see p. 533). The occurrence of the latter was associated with differences in light intensity. Hunt (1925) deduced that phytoplankton was in all probability the chief ultimate source of supply of organic matter to the bottom in the Plymouth region.

It should be noted from the above list that protozoa, bacteria and small diatoms were hardly ever encountered in direct searching, even though alive and active and found by other means to be very numerous. Petersen and Boysen Jensen (1911) stated as a result of direct microscopic examination that "Of living organisms apart from bacteria and higher animals there are but few; one or two living bottom diatoms were met with." Krogh and Spärck

(1936) gave values for the numbers of "infusoria" found by direct searching, which from the results obtained by another method at Rame would appear to be far too small, but perhaps they refer only to very large ciliates. Investigators of the soil protozoan population have found direct searching to be valueless, as protozoa have a definite affinity for the soil particles, adhering closely to them so that they are hardly ever seen. The relatively enormous number of silt particles compared with the living organisms would also tend to make such a method very unsatisfactory for assessing the numbers present. The number would change appreciably before many samples could be counted.

The Microbenthos

A study has been started of the types, numbers and distribution of the micro-organisms and the quantity of living matter present in the surface layers of the mud deposit at the Rame station. A little information about the part played by these micro-organisms in the food cycle is here collected: it has been shown that indigenous photosynthetic organisms are present on the sea bottom; the importance of bacteria as converters of organic debris is known chiefly from the work of Waksman *et al.* and ZoBell *et al.*; data are here given on the feeding habits of some of the protozoa of the Rame microbenthos. The ways in which bacterial and protozoan populations interact, and particularly the influence of protozoa on bacterial activities associated with the regeneration of nutrient salts, are important subjects which have yet to be explored in marine habitats.

There has as yet been little ecological work on populations of micro-organisms treating them as communities rather than as collections of individuals. The most extensive work of the type indicated above has been carried out by Cutler, Crump & Sandon and others at Rothamsted on soil populations. Special attention was there paid to the determination of the numbers of organisms present, to fluctuations in abundance, and the influence of protozoa on bacterial activities affecting soil fertility. Picken (1937) described the structure of some freshwater protozoan communities, laying stress on the interrelationships of the organisms with each other on the basis of food chains, and their dependence on the structure of the habitat. In a distributional study of the microscopic benthic fauna of a Michigan lake, Moore (1939) noted the effects of depth, type of deposit and of seasonal changes in the oxygen concentration on the numbers and species of such protozoa as were observed by direct search.

In marine habitats, the nannoplankton was investigated extensively by Lohmann (1908, 1911), who determined the numbers and volumes of all the minute organisms of sizes comparable with the microbenthos, and placed them in relationship with the rest of the plankton. The predatory activities of protozoa in the water are held by Waksman & Hotchkiss (1937) and others to be important in limiting the numbers of bacteria normally found in suspension in the sea. Lackey (1936) made qualitative studies of the protozoa from the water and from the bottom and discussed their possible roles, pointing out that protozoa have an important function in the turn-over of organic matter. For example, a dead copepod is soon invaded by bacteria, followed by ciliates, which, after escape from the carapace, may serve as food particles of convenient size for metazoan filter feeders. 'The holozoic and saprophytic forms attack organic debris in suspension and constantly convert it to protoplasm in part and accomplish its mineralization in part. If they do not compare with the bacteria in numbers, their size is a compensation for this.' He also suggested that the smaller and presumably saprozoic flagellates may act as 'food condensers' for the larger forms.

The only members of the microbenthos which have so far been investigated in detail are the bacteria. Lloyd (1931), Waksman *et al.* (1933 *et seq.*) and ZoBell *et al.* (1936 *et seq.*) have investigated bacterial numbers, distribution and physiological types in sea water and in bottom deposits. They have been concerned with the bacteria and with the net effects in nutrient salt regeneration and have not investigated the influence of protozoa in the benthic community.

The possibility that marine fungi might be present on the bottom was investigated by Sparrow (1937), who found no typically marine forms there, and stated that it was doubtful if fungi are present in a trophic state or take any part in the decomposition of organic detritus.

An attempt is here made to fill in one of the gaps in our knowledge of the organisms of the sea bottom, and investigations have been made in detail on the protozoa and algae present on the bottom. Some work on bacteria has been included.

Census Methods for Micro-organisms

Routine Procedure

The routine procedure for obtaining a sample and for its subdivision was as follows. The core sampling apparatus used has been described on p. 520. A sterile glass tube was fitted for taking each sample and at least ten good cores were obtained. Since the surface area of each core was 2·54 cm.^{2}, census data of the micro- and meiobenthos (expressed as numbers per gram of dry deposit or numbers per unit area) are from mud derived from an area of 25·4 cm.^{2}, unless otherwise stated. Full precautions were taken against contamination by extraneous organisms in the laboratory and on board ship. A flow of water through the tube during its descent was, however, inevitable; but despite this, calculations showed that there could have been no significant contamination by organisms from the water. No contamination was possible during ascent, with the glass tube plugged at the bottom by the core of mud (the bottom of which was discarded) and closed at the top by the valve. Immediately on being brought on deck, sterile corks were put in both ends of the tube. The cores were transported vertically in an ice box and used immediately on arrival in the laboratory (2–3 hr. after being taken). The supernatant water was siphoned off each in turn to just above the surface of the mud, and the level of $\frac{1}{2}$ cm. down the core was marked with a rubber band. Sterile water was then added and the top $\frac{1}{2}$ cm. of mud with this water siphoned over into a sterile burette, where the volume was measured before discharge into a flask. The open ends of the sample tube and the flask were protected by sheet rubber hoods. In this way none of the rather fluid surface layer was lost and contamination in the whole process was found experimentally to be quite negligible. Any organisms migrating up or thrown into suspension just above the surface of the mud were included with those living in the surface layer. The suspension was used first for setting up the bacterial count and then for the protozoan and algal census. An aliquot sample of 50 c.c. was also taken to ascertain the dry weight of the mud. The total volume of mud and water from ten cores was usually about 350 c.c., containing about $\frac{1}{25}$ g. of mud per cubic centimetre of suspension.

Census Method for Bacteria

Work on bacteria was restricted to assessing the number of aerobes present (without further distinction of types), and to evaluating the bacterial contribution to the total bulk of living matter. Bacteria were plated from dilutions of approximately 1/2500 and 1/25,000. After a comparison of several media, including those used by Waksman *et al.* (1933) and ZoBell & Anderson (1936), it was found that the medium giving the highest counts was that used by Waksman with the interesting addition of a trace of iron:

Sea water	1000 c.c.	K_2HPO_4	0·05 g.
Bactopeptone	1 g.	$FeCl_3$ (anhyd.)	0·0125 g.
Glucose	1 g.	Agar	15 g.

The optimum *p*H was found to be 7·5 (as shown by cresol red corrected for salt error). The suspension was shaken for 3 min. immediately before plating to disperse aggregations of bacteria. Incubation was at room temperature and the plates were observed after 14 days, by which time significant differences were easily detected. There are so many factors slightly affecting the numbers of bacterial colonies counted that it was considered safe only to compare plates set up on the same day.

The plate count represents only a small and not necessarily a constant fraction of the total number of bacteria present in the habitat, owing to the impossibility of finding a medium or conditions in which all types of bacteria will develop. Direct count methods were tried but found inapplicable to a marine sediment. Data obtained by workers on other habitats (soil, fresh water and sea water) show that their plate counts represent something of the order of one-hundredth of the numbers they obtained by direct counts. The latter, however, include dead as well as living bacteria.

Census Method for Protozoa and Algae

A dilution culture method, modified from that used by soil microbiologists (see Cutler, 1920; Cutler *et al.* 1922), was used to count the protozoa and algae. The suspension of mud was diluted by stages and 1 c.c. samples plated out into Petri dishes containing a suitable medium. The dilution stages were in orders of two or four with, respectively, one or two plates at each dilution. A range of dilutions from approximately 1/25 to 1/102,400 was necessary to include all the protozoa and to 1/6,553,600 to cover all the diatoms. The medium which was found to encourage the greatest variety of species was made up as follows:

Sea water	1000 c.c.	Cystine or methyonine	10 mg.
$NaNO_3$	0·1 g.	Gluconic acid	2 mg.
Na_2HPO_4	0·02 g.	$Fe^{\cdots}$	0·1 mg.
Sea mud extract	100 c.c.	$Mn^{\cdot\cdot}$	0·02 mg.

The nitrate and phosphate are necessary for plant growth. The sea-mud extract was made by autoclaving mud from the Rame habitat, in a similar way to the soil extract used by Gross (1937). The last four ingredients had recently been found advantageous to the growth of diatoms by Harvey (1939). This medium was found to give a much greater variety of species than media richer in organic matter, in which bacterial growth was liable to be excessive. By keeping the cultures in a dull light at 13° C., a satisfactory gradual succession of species was obtained. The plates were examined at weekly intervals, samples being removed with a fine sterile pipette for recognition of species.

From the presence or absence of any particular species down the dilution series the number of positive plates for that species is recorded, and from this the original concentration of the species can be calculated (see Cutler *et al.* 1922, table iii, p. 341). It is assumed (and there is definite evidence in some cases) that any single organism is capable of giving rise to a population which on examination of the dishes will be detected. However, with a wide variety of species in mixed culture (see p. 528) each of the individuals of a species in a dish sometimes inevitably fails to multiply. Some allowance can be made for such gaps from a table[1] of the probabilities of occurrence of the various types of end-point in the dilution series. For estimates of the total population, the filling in of obvious culture gaps is essential. These estimates are in any case *minimal* values for the population, and with standardized conditions and technique, valid *comparisons* can be made between parallel series from different places and between successive samples from the same place. The significance or otherwise of the differences obtained is tested on the number of positive plates, reliance being placed on those species which show few or no culture gaps. A difference of at least three plates is significant for any single species, and therefore where such a difference occurred, the numbers of that species per gram of deposit were also significantly different. Since groups of species compared showed the same tendency for high or low figures these smaller grouped differences were usually significant. The method was tested as follows: it was used to count a ciliate population of known density, and in each of two tests with three parallel dilution series the number of positive plates was within one plate of the expected number. In making two parallel estimations of the same natural mixed population, technical variations were found to be insignificant.

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[1] A table kindly prepared for my use by Mr G. M. Spooner.

In the tables on pp. 530–533, the numbers are given per gram of dry mud. The volume of an organism has been calculated from its dimensions, using individuals of average size found in culture. The volumes of all species have been summed to give the volume of protoplasm (in cubic millimetres) contributed by a group such as ciliates or amoebae to the total bulk of living matter forming the microbenthos.

Limitations of Methods

A clear distinction must be drawn between the value of these census methods for comparative purposes and for giving absolute numbers. Although they cannot be made very accurate, with standardized technique they are adequate for comparative work when large differences are involved. The absolute number of living bacteria lies between the number deduced from the plate count and approximately 100 times that number. The census method used for the other micro-organisms includes both the trophic forms and any resting stages there may happen to be on the bottom, but it is a minimal value for the total number of organisms because an unknown number of species may have failed to develop under the conditions provided. At present there is no more adequate method available.

A comparison of direct and culture counts, made on a sand deposit, illustrated the difficulties and limitations of both census methods. For protozoa and small diatoms the culture method gave much higher figures; but only a few of the larger planktonic diatoms seen in direct search had responded to culture conditions because some of the vegetative cells were too moribund to reproduce; others may have found the conditions or the media unsuitable. There was, however, some development of resting spores. By culturing a sample of detritus freshly deposited from sea water into a glass dish, in which only a few species were found by direct observation, Lackey (1936) obtained several other species of protozoa and algae by excystment. One must therefore be very cautious in discussing results obtained only by culture methods, but for members of the microbenthos, direct examination has led to erroneous results (see p. 522).

Types of Organisms found and their Habits

Bacteria

Rods and cocci were common, including motile forms. Measurements indicated that the average volume was about 1 cu. μ. This figure was used by ZoBell & Feltham (1938) and Baier (1935) for calculations of an available food supply. The physiological types have been investigated by several workers, recently by Waksman *et al.*, ZoBell *et al.* and Lloyd. ZoBell (1939) reviewed the subject of bacteria in deposits.

Protozoa and Algae

The species of protozoa and naviculoid diatoms have in most cases been known by numbers. Taxonomic studies have been omitted, as these would have very much enlarged the scope of the work. Some ten species of flagellates, three species of amoebae, two of ciliates and about twenty species of diatoms occurred regularly in the cultures and showed fairly consistant behaviour. It was usual for about 40–50 species in all to be present in the mixed cultures of the dilution series. To this must be added the bacteria developing in these cultures. It is therefore not surprising that irregular gaps in the dilution series, due to culture failure, were apt to occur, especially among forms developing late.

The general sequence of events in one culture dish was as follows:

(1) Bacteria developed rapidly during the first week.

(2) The small colourless flagellates were present at the end of the first week—spp. II, IV, V (see p. 529 and Fig. 2).

(3) These were soon followed by another group of colourless flagellates—spp. VI, VII, XI, XII.

(4) Meanwhile planktonic diatoms were rapidly increasing and reached a maximum about the second or third week according to the light intensity. They then decayed.

(5) This gave rise to abundant organic matter, causing a further increase in the bacterial population. Most of the first group of small colourless flagellates had by this time died down, but not infrequently spp. II, IV and V persisted and were very abundant.

(6) Another group of flagellates, some containing coloured food vacuoles appeared about the third week. These (spp. I, XX, X and others) were rather larger than the first group.

(7) Amoebae were occasionally found early in the month, but they did not become abundant until there was plenty of plant debris. Some appeared to feed directly on the plant debris while others contained bacteria in their food vacuoles.

(8) During the decay of the planktonic diatoms, naviculoid diatoms and species of *Nitzschia* and *Gyrosigma* developed and usually remained active until well after the fourth week. In the winter months with low light intensity these bottom diatoms were conspicuous earlier in the sequence. Probably their compensation point (the light intensity at which the rate of photosynthesis and respiration are equal) is much lower than that of the planktonic forms.

(9) Ciliates only occurred at the more concentrated end of the series. They usually appeared early and were persistent.

(10) If coloured flagellates occurred, it was towards the end of the month. These were probably members of the nannoplankton.

(11) After prolonged standing occasional red, green or brown algae developed, presumably from spores of littoral species.

Under the term 'bottom diatoms' are included pennate species, mainly naviculoids, which are not normally part of the net plankton. Varieties of *Nitzschia closterium* have been present so constantly that they have been included, though possibly they should be regarded as tychopelagic. Other tychopelagic forms, such as *Paralia sulcata* have been included with the planktonic diatoms. The distinction drawn has been partly one of convenience, separating those species which develop early in the cultures and were generally found in suspension from those which developed more slowly and usually crawled on the bottom. Members of this latter group were the only diatoms to develop under conditions of low light intensity. On account of its good growth in poor illumination, it is reasonable to include *N. closterium* with the bottom forms. The following species of planktonic diatoms occurred frequently in the cultures: *Thalassiosira gravida*, *Th. nana*, *Sceletonema costatum*, *Chaetoceros* spp., *Asterionella japonica*, etc.; the *Rhizosolenia* spp. were for the most part conspicuous by their absence. These species of planktonic diatoms did not reflect the changes in species seen in net plankton hauls made regularly over the Rame station. This discrepancy may be due to culture conditions, to the development of some species from resting spores independently of the season, to the hardiness of the vegetative cells of a few species, or possibly to some selective consumption by the zooplankton.

Some of the organisms mentioned above closely resembled species previously described, but identification is tentative. Others, while they cannot be named, were readily recognized by their characteristic behaviour and simple morphology:

FLAGELLATES.

II resembles *Bodo angustus* 8–12 μ long, cf. Lemmermann (1914).
IV is a *Monas* sp. 4–6 μ long.
V is a *Bodo* sp. 4–5 μ long.
VI is a Choanflagellate 4 μ long.
VII resembles *Rhynchomonas nasuta* 5 μ long, cf. Griessmann (1914).
XI resembles *Rhynchomonas mutabilis* 10 μ long, cf. Griessmann (1914).
XII is a *Bodo* sp. 2–3 μ long.
I is a *Bodo* sp. 16 μ long, flattened.
X resembles *Telonema subtilis* 6–8 μ long, cf. Griessmann (1914).

AMOEBAE. Normal types, 5–10 and 15–30 μ diameter (excluding pseudopodia) and *limax* type *ca.* 16 μ long.

HELIOZOA. An occasional species, *ca.* 5 μ diameter.

CILIATES. *Uronema marinum*, 20–25 μ long, cf. Kahl (1935).
Lembus pusillus, 20–30 μ long, cf. Kahl (1935).
Trochilia sp., 16–20 μ long, cf. Kahl (1935).
Mesodinium pulex, 15–20 μ long, cf. Kahl (1935).
Hypotrichous species up to 50 μ long.

Several of these species are by no means restricted to the bottom habitat, they have been cultured from water samples and some have been found on a sandy shore. From Lackey's list of protozoa, classified according to habitat—floating, attached or in debris and on the bottom—it seems possible that some of the protozoan species may be only casual inhabitants of the bottom. In culture many of the flagellates and ciliates swim freely in the water, and it should not be supposed that individuals are necessarily confined to the deposit under natural conditions.

Results of Culture Census of the Microbenthos

The following tables summarize the results[1] of sampling between January and June 1939. Samples taken previously, while the technique was being standardized, are (so far as can be judged) in reasonable agreement with these later results. The comparisons given in Tables I–IV are based on minimal numbers, and the figures always indicate the order of magnitude of the numbers and quantities involved rather than their exact values. Of these organisms the bacteria, protozoa and some of the bottom diatoms may be regarded as constituting the true microbenthos. The planktonic diatoms are best regarded as a part, and probably only a small part, of the deposition on the bottom, which with detritus forms the main food supply of the benthos. Attention is again drawn to the limitations of the methods here employed (see p. 527). The results are still tentative, as there has not been sufficient opportunity to confirm any of the conclusions, which for the present are made on single or relatively few estimations.

Horizontal Distribution

Table I records the results of an attempt to investigate the local horizontal variations in numbers. The samples were taken on 24 January. Numbers per gram of dry mud from the top $\frac{1}{2}$ cm. layer from the surface of five tubes are compared with those from another five tubes. The cores were grouped at random from a set of samples taken at the Rame station. Some part of the difference in the total number of bacteria and of protozoa per gram may be due to the fact that rather less mud was taken off the surface of the cores 6–10 than off cores 1–5, there being probably some vertical gradation in abundance. Even if bacteria and protozoa were entirely concentrated in the extreme surface, irregularities of depth of sampling could not make the numbers per gram in cores 6–10 as much as twice those in 1–5. Therefore there must be very considerable local variations, particularly among flagellates and bacteria. Diatoms are much more evenly distributed; this fairly even distribution has been confirmed in another count of diatoms alone. In subsequent comparisons

TABLE I. HORIZONTAL VARIATION

	Cores 1–5			Cores 6–10		
	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud
Bacteria (plate count)	?	345,000	0·0003	?	2,050,000	0·002
Ciliates	2	15	0·0001	2	75	0·0003
Amoebae	4	2,335	0·0013	4	6,160	0·005
Flagellates	17	14,900	0·0028	13	79,080	0·015
Bottom diatoms	11	16,440	0·0046	10	46,270	0·0049
Planktonic diatoms and algae	17	21,140	0·0083	18	49,000	0·007
Total vol. of living matter			0·017 c.mm			0·035 c.mm.

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[1] Full tables, illustrating the method of calculating the results (see p. 526), are available in a thesis placed in the Cambridge University Library and in the Library of the Marine Biological Association.

ten cores were mixed, and it is hoped that local irregularities of distribution were thereby eliminated. Owing to difficulties of sampling it was not practicable regularly to obtain more cores.

Vertical Distribution

Table II summarizes the results of a comparison of the micro-organisms found in the surface ½ cm. layer of mud at the Rame station with those found at a depth of 2·5–3·0 cm. in the same cores. The sample was taken on 27 April.

Table II. Vertical Variation

	Surface, 0–0·5 cm.			Deeper layer, 2·5–3·0 cm.		
	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud	No. of spp.	No. per g. dry mud	Vol. of protoplasm in c.mm. per g. of dry mud
Bacteria (plate count)	? 12	710,000	0·0007	? 12	145,000	0·00015
Ciliates	2	12	0·00003	1	7	0·00007
Amoebae	3	6,850	0·0005	2	230	0·0003
Flagellates	15	49,800	0·0034	10	2,840	0·0018*
Total protozoa		(56,660)			(3,080)	
Bottom diatoms	13	118,090	0·012	8	34,160	0·0025
Planktonic diatoms and algae	13	346,420	0·33	12	23,940	0·0079
Total vol. of living matter			0·35 c.mm.			0·013 c.mm.

* The full tables show that the volume of flagellate protoplasm at the lower depth is almost entirely due to the presence of relatively few large dinoflagellates belonging to a single species (4% of the total number of flagellates giving rise to 90% of the volume). It is most unlikely that this species was in an active state when buried. If it were omitted from the list or a volume smaller than that of the active vegetative cell used in the calculations, the volume of flagellate protoplasm in the deeper layer would be much smaller than that in the surface layer. This is in better agreement with the numerical differences.

The surface layer is seen to be much richer in all groups than is the layer deeper in the core. It is obvious that planktonic diatoms contribute by far the greatest bulk to the total volume of living matter, especially on the surface. It is this rich layer which is of particular importance to many detritus eaters.

Seasonal Variations

Seasonal variations in the numbers of diatoms present in the surface layer were followed from winter to summer conditions. Variations in the numbers of protozoa could not be considered as seasonal variations since there were obviously large local variations, the greatest difference between samples being that shown in Table I. It is possible that there may be short-period fluctuations in numbers of protozoa and bacteria similar to those found in the soil by Cutler *et al.* (1922), but no attempt was made to investigate this point.

Separate stages in the changes in abundance from winter to spring and summer conditions are not significant, but the general drift is significant. There is a fourfold increase in numbers of bottom diatoms, and they are of considerable importance in the winter. Owing to the very great rise in the number and the greater bulk of planktonic species, these become overwhelmingly important in the spring and summer.

Hunt stated that bottom diatoms 'have been found in sufficient quantity in the stomachs of various animals to suggest that they contribute a very important ultimate food factor for the animals of such a region'. The lack of knowledge as to their abundance and seasonal variation in numbers has now to some extent been remedied. Owing to the lack of agreement between the planktonic species (see p. 529) occurring on the bottom and in the water, a detailed comparison cannot be made between the numbers deposited and the phytoplankton crop.

Table III. Seasonal Variations in the Numbers of Diatoms

All samples from the surface layer at the Rame station.

Date 1939		Planktonic diatoms (Pl.)				Bottom diatoms (B.)				Total vol. of protoplasm per g.	Ratio of vol. Pl. : B.
		No. of spp.	+ve pl.	No. per g.	Vol. per g.	No. of spp.	+ve pl.	No. per g.	Vol. per g.		
24 Jan.	*a*	13	52	26,000	0·0083	11	54	26,000	0·0046	0·013	1·7 : 1
	b	15	51	16,000		8	34	6,400			
	c	15	55	48,000	0·0076	10	42	46,000	0·0049	0·013	
6 Mar.	*d*	15	74	71,000	0·026	12	46	14,000	0·0042	0·042	8 : 1
	e	16	67	178,000	0·048	10	49	11,000	0·0049		
16 Mar.	*f*	14	60	196,000	0·030	12	70	99,000	0·015	0·045	2 : 1
3 Apr.	*g*	17	69	112,000	0·19	12	66	84,000	0·018	0·21	10 : 1
27 Apr.	*h*	13	80	364,000	0·33	14	65	118,000	0·012	0·34	33 : 1
31 May	*i*	15	75	604,000	1·28	11	54	156,000	0·019	1·30	67 : 1

a and *b*, each from five cores (see Table I).
c, from an independent culture series using mud from *a* and *b*, from ten cores.
d and *e*, each from five cores. *f*–*i*, each from ten cores.

Regional Distribution

Samples were collected from different types of deposit and from various depths. A comparison was made between a Rame mud sample taken on 16 March and an Eddystone shell-gravel sample taken on 14 March, so that the cultures were running simultaneously in the same light intensity. The coarse deposit was collected with the Hunt vacuum grab, which takes an unstratified sample from an area of about 42 cm.^{2} and to a depth of about 2 cm. on sands and gravels. Figures are given per gram of total deposit, as in the probable absence of a distinct surface layer this is the form most comparable with the Rame figures. No bacterial estimation was made, because the Hunt grab in its present design is unsuitable for bacterial sampling. The number of bacteria per gram of total deposit is likely to be low in shell-gravel.

All groups are significantly less abundant in the shell-gravel than in the mud. A deposit-eating member of the shell-gravel fauna would, however, probably ingest only the fine particles; the proportion of living matter in this small silt fraction is quite as high as at Rame. Smith (1932) recorded the presence of animals feeding both selectively and non-selectively on bottom material and also, of course, of carnivores. Suspension feeders are, however, the members of the macrofauna most common on the Eddystone shell-gravels. The figures in Table IV indicate that there is an appreciable deposition of planktonic diatoms, and so presumably of other organic matter; water movement over the bottom will keep some of this material in suspension. It is possible that some of the organisms here recorded per gram of deposit were actually in suspension immediately over the bottom and brought up with the supernatant water which is unavoidably collected by the vacuum grab.

TABLE IV. REGIONAL VARIATION IN DIFFERENT TYPES OF DEPOSIT

	Rame mud (regular station), sample taken 16 March			Eddystone shell-gravel, sample taken 14 March		
	No. of spp.	No. per g. of dry mud	Vol. of protoplasm in c.mm. per g. dry mud	No. of spp.	No. per g. total deposit	Vol. of protoplasm in c.mm. per g. dry deposit
Ciliates	2	30	0·0002	2	8	0·00003
Amoebae	3	5,680	0·0033	3	680	0·00036
Flagellates	12	11,614	0·0013	6	600	0·000022
Bottom diatoms	12	99,470	0·015	11	7,680	0·007
Planktonic diatoms and algae	16	196,000	0·030	6	38,590	0·006
Total vol. of living matter, excluding bacteria, per g. total deposit			0·050 c.mm.			0·013 c.mm.

A few other samples, though not directly comparable with the preceding results, gave a preliminary indication of the distribution of diatoms on the bottom. Planktonic diatoms were much less frequent on coarser deposits seawards from the Rame station, and they were not found in sand on the shore at Wembury nor in the estuarine mud at Laira. Naviculoid diatoms had a surprisingly wide distribution in the sublittoral zone, at least during the summer. For example, *Cocconeis* sp. occurred quite abundantly with several other species as well, on the sandy bottom at Station E 1, 20 miles from the land and at a depth of 72 m., but under clearer water than at Rame. In a sample[1] taken at the mouth of the English Channel (at 49° 9′ N., 6° 7′ W.) from a depth of 113 m. no planktonic diatoms were found, only one large naviculoid diatom species developed in culture and a few other moribund cells of tychopelagic and bottom species were seen. Owing to the unavoidable delay of 6 days after sampling before examination and the setting up of cultures it is difficult to be certain of the reason for the dearth of plants, but it seems probable that the limit of active plant life on the bottom had nearly, if not quite, been reached at this station. The exact significance of the commonly accepted idea of a lighted benthic zone down to a depth of about 100 fm. (the sublittoral zone) needs more thorough investigation.

The Quantity of Microbenthos relative to the total Organic Matter in the Deposit

In order to assess the contribution that the microbenthos makes to the total food supply available to the detritus-eating macrofauna an estimate would have to be made of the absolute population density. Since this is at present impossible, an arbitrary method has been employed of obtaining from the culture census figures something more nearly approaching a maximal value for the population. From this extremely rough approximation it has been estimated that, if the organic carbon at Rame be taken as 1 g. in 100 g. dry mud, then in the surface $\frac{1}{2}$ cm. layer only about 0·3–0·03 % of this carbon is derived from bacteria, protozoa and bottom diatoms; at least a further 2·5–0·015 % comes from recently deposited and still living planktonic diatoms, according to the season. At a depth of 2·5–3·0 cm. below the mud surface, a single sample indicated that only about one-fifth of the volume of microbenthos was present compared with that at the surface, and probably there would be even smaller quantities at greater depths.

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[1] Kindly taken for me by Mr G. A. Steven.

Comparison with other Habitats.

Some of these results are supported by comparison with those of other workers. Data from the western Atlantic coast given by Reuszer (1933) show the richness of the bacterial population in the extreme surface layer of a mud deposit compared with the numbers present at a depth of 2·5 cm. in the mud and with those found in the top 2·5 cm. of a sand deposit. The organic content of the mud was considerably greater than that at Rame, but despite this the numbers of bacteria were of the same order in the two habitats. Reuszer found up to a hundredfold variation between the bacterial content of different cores and concluded that dense aggregations of bacteria might be associated with recently deposited organic matter. Lackey (1936) gave figures which showed the richness of the protozoan fauna associated with freshly deposited detritus, and in stressing the importance of the surface layer of mud for the activities of the protozoa, stated that the surface of mud cores from shallow water contained 'an abundance of flagellates and ciliates', whereas samples from a sandy bottom yielded few protozoa. Remane (1933), on the other hand, in comparing the number of species of ciliates in sand and mud deposits in the shallow waters of Kiel Bay (maximum depth 20 m.) found very few ciliates in the mud. It is, however, probable that the black mud there was of a stickier consistency than that at Rame. The sand habitat in Kiel Bay contained many species of ciliates, but the population density is unknown. On this sand there was a rich flora of bottom diatoms.

In freshwater deposits Moore (1939) found by direct examination that protozoa were most numerous in the top centimetre of the cores and that the numbers decreased rapidly to extinction at about 5 cm. below the surface on 'muck' bottoms. Pennak (1940) made brief mention of the micro-organisms found in a sandy beach of a large freshwater lake: in general composition and in the importance of ciliates, coloured flagellates and also rotifers, there was considerable similarity to the microbenthic community found in sand on the sea shore at Wembury. Micro-organisms present in arable soil at Rothamsted (see Cutler *et al.* 1922) were more numerous than in the Rame deposit, a difference possibly associated with the smaller amount of available organic matter in the sea bottom. Groups occurred in the same order of abundance (bacteria, flagellates, amoebae and ciliates) in both habitats, but when account is taken of size the amoebae were possibly relatively more important than flagellates in the soil, whereas there was more nearly an equality of volumes of these two groups in the Rame deposit.

Food Relationships

Diatoms have been shown to constitute a large part of the microbenthic community. It is important to consider whether or not they are able to photosynthesize when on the bottom. Jenkin (1937) found that the compensation point for *Coscinodiscus excentricus* (a planktonic diatom requiring low light intensity) was at 45 m. in July near the Eddystone, over cleaner, sandier ground than the Rame station. In Jenkin's experiments the light intensity at 45 m. was about 0·5 % of that at the surface. Poole & Atkins (1929) found the light intensity very close to the sandy bottom, at 70 m. at station E 1, in March, April and May to be respectively 0·016, 0·098 and 0·121 % of that at the surface; on this deposit (sampled in May) a small naviculoid, *Cocconeis* sp., was found to be abundant. In the immediate neighbourhood of the bottom over mud there must be a rapid decrease in light intensity. It is therefore probable that there is little or no production by planktonic diatoms in the vicinity of the bottom at the Rame station. Since bottom pennate diatoms have a lower compensation point than planktonic species it seems probable that sufficient photosynthesis for net production can be carried on by bottom diatoms when lying on the extreme surface and to a greater extent when they are washed into suspension. These diatoms may be buried by falling debris or by disturbances of the bottom due to the epifauna or burrowers, but since most bottom diatoms are motile some will regain the surface. Small coloured flagellates have also been found on the bottom, but it is thought that they are really members of the nannoplankton, and nothing is known of their light requirements.

Some deductions concerning the food habits of protozoa, when under culture conditions, can be made from observations on the dishes of the dilution series and from other cultures. It was observed for instance that both *Uronema marinum* and *Lembus pusillus* throve in pure cultures on a diet of bacteria, but that in the mixed cultures of the dilution series, while *Uronema marinum* continued to feed almost exclusively on bacteria, *Lembus pusillus* nearly always had coloured food vacuoles, containing the remains of diatoms or coloured flagellates. Thus it seems that these two ciliates differ in their food preferences. To what extent such preferences would be apparent in the natural environment would be dependent on the relative abundance of bacterial and plant food. Both ciliates probably ingest detritus. A large *Bodo* species was observed on one occasion to have been ingesting numerous small chlamydomonads, but generally the plant-eating flagellates and also ciliates and amoebae were dependent on diatoms already partially disintegrated by autolysis or bacterial action and flagellates have been observed ingesting chloroplasts inside frustules of decaying diatoms. Under natural conditions it is probable that these plant-eating forms are capable of subsisting on a variety of diets; possibly they require more abundant organic particles than do the smaller flagellates. The small flagellates mentioned in stages (2) and (3) of the culture series (p. 528) were observed to contain particulate food; they tended to remain on or near the bottom surface of the dish unless there was dead organic material in suspension. Some clearly showed aggregation round organic particles. It is thought that they are chiefly bacterial feeders and probably also saprophytic. Sandon (1932), while not doubting the possibility of saprophytic nutrition among the Protomastigina, does not regard it as definitely proved. Some of the colourless euglenids seen occasionally in these cultures may well be saprophytic. As far as is known amoebae always depend on the ingestion of solid food; the way in which they grazed the film of bacteria on the bottom of a Petri dish made patches of amoebae easy to detect with a dissecting microscope. Soil amoebae are known to show distinct preferences for various types of bacteria. Singh (1941) has demonstrated such preferences in both pure mixed culture and in sterilized soil, but has found that the amoebae are not so destructive of edible species in the soil cultures. This further illustrates that predator-prey relationships may well be quite different when 'refuges' are present, as was shown by Gause (1934).

The interaction of protozoa and bacteria may be of importance in several ways in the turn-over of organic matter in a deposit. The predatory activities of amoebae and ciliates may exert a marked influence on the bacterial numbers, depending on the relative volumes and rates of reproduction of the predators and prey. These predatory activities, if excessive, would decimate the bacterial population. They may, however, only be sufficient to keep the bacterial numbers at or near such a level that each bacterial cell exhibits its maximum chemical efficiency; such an effect has been found by Cutler & Crump (1929) and by Meiklejohn (1930, 1932) with pure cultures of bacteria and soil amoebae or ciliates. Protozoa and bacteria may compete for available organic matter, either dissolved or particulate (see Fig. 2, legend).

Great care must be exercised in deducing from results of culture experiments any relationships in the natural habitat. In cultures here set up no attempt was made to reproduce exactly the natural conditions. A culture dish of the dilution series was an isolated habitat with only very sparse mud particles. It usually had a large initial supply of organic food followed by the naturally produced abundant plant food, which resulted from a light intensity far in excess of that in the natural habitat. When small doses of organic food were added at weekly intervals the protozoan culture succession was delayed, though the additions were not accurately adjusted to maintain a balanced population. When cultures were grown under much reduced light intensities, thus approaching more nearly the natural conditions of the habitat, only a few

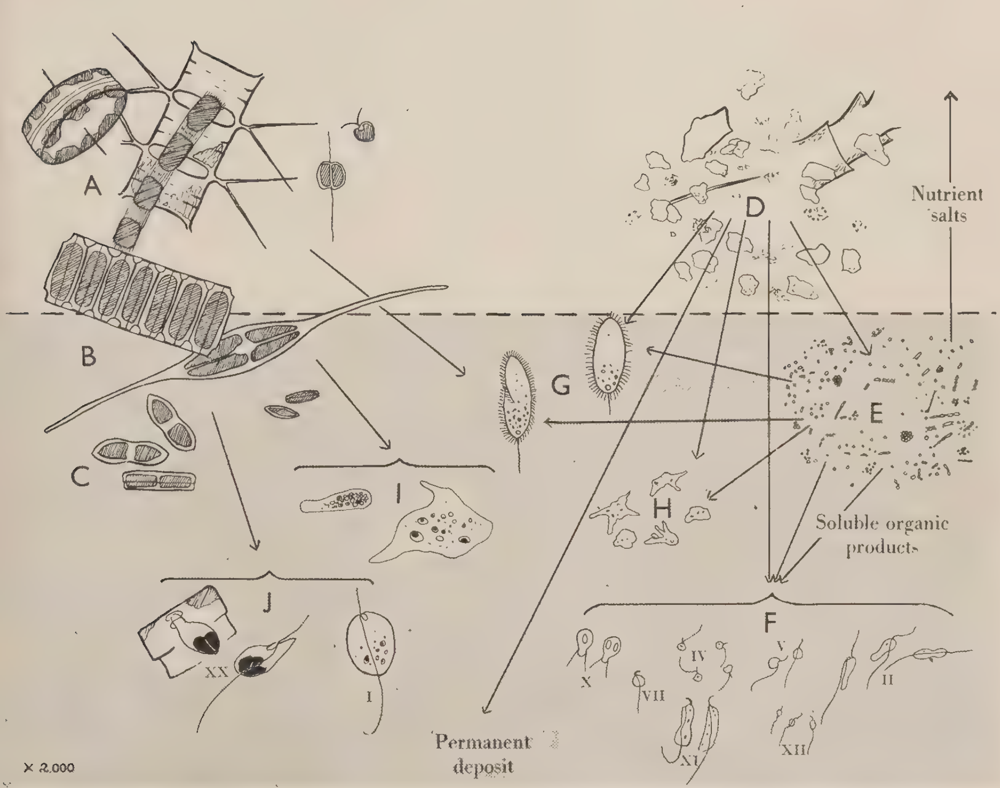

Fig. 2. Food chains in the microbenthos. This diagram represents probable food relationships between supplies dropping from the water and organisms in the surface layer of the mud. The organisms represented are mentioned on pp. 528 and 529 and are drawn to scale (1 mm. $= 2\,\mu$). Relative abundance cannot be adequately represented. A. Phytoplankton—diatoms and coloured nannoplanktonic flagellates. B. Tychopelagic diatoms. C. Bottom naviculoid diatoms. D. Detritus—animal and plant remains and faecal material already disintegrated and partly decomposed in the water. E. Bacteria—by means of extracellular enzymes they utilize detritus, converting part into their own protoplasm, liberating nutrient salts to the water, and freeing some soluble organic matter which may be used by other bacteria or by flagellates. F. Small colourless flagellates—they ingest bacteria and possibly detritus and may also live saprophytically. G. Ciliates—*Uronema marinum* is known in culture to digest bacteria and probably also takes detritus. *Lembus pusillus* ingests bacteria and if plant remains are abundant, has many coloured food vacuoles. H. Small amoebae—bacterial feeders, probably also ingesting detritus. I. Amoebae—a large normal and a limax type are often seen with coloured food vacuoles, they probably also take other types of food. J. Large colourless flagellates—with coloured food vacuoles, one is shown inside a dead diatom. Plant-eating protozoa cannot ingest whole diatoms, but depend on fragments produced by autolysis and bacterial action. The resistant residues of detritus are permanently buried in the mud.

naviculoid diatoms developed and the growth of planktonic species was prevented.

It is clear that the groups of the microbenthos are by no means independent of each other. Only the broadest outlines of qualitative relationships in the habitat are suggested in Fig. 2. A determination of the relative abundance (see pp. 530–533) and of the rates of reproduction of the various species and characteristics of their behaviour under natural conditions are essential before further deductions can be made. It is suggested, however, that the possible influence of protozoa in the nitrogen cycle or in regeneration of phosphates in the sea should not be overlooked.

Summary of the Activities of the Microbenthos

It is helpful here to summarize the qualitative knowledge of the activities of the microbenthos in the turn-over of organic matter in a sub-littoral habitat. These activities may be subdivided as follows:

(1) *Production* of organic matter from inorganic compounds.

  (*a*) Chemosynthesis by bacteria is known to play a part on the bottom (see ZoBell, 1939).

  (*b*) Photosynthesis. The activity of the bottom diatoms will obviously depend on the light intensity, varying with the season and with the depth and clearness of the water. It is thought probable that there is some production, at least in the spring and summer, on the bottom at the Rame station.

(2) *Conversion* of organic matter from one form to another is chiefly carried out by bacteria. They change indigestible forms such as chitin (see ZoBell & Rittenberg, 1938) or celluloses into more digestible bacterial protoplasm. ZoBell & Feltham (1938) and MacGinitie (1932) have proved that some bacteria may serve as food for metazoa, and it is known that they are an important food for protozoa. The bacteria, however, also utilize the more available fractions of the organic detritus in their own metabolism, leaving indigestible residues known as marine humus (Waksman, 1933).

(3) *Accumulation* of soluble organic matter as protoplasm by bacteria and saprozoic protozoa is certainly an advantageous process for the metazoa. On the other hand, the concentration of small food particles by protozoa, though of possible advantage to metazoa obtaining food by filtration of large volumes of water, is an extravagant process in a mud habitat, because the protozoa themselves consume some of the organic matter which they accumulate and a deposit feeder ingests all small particles in whatever size or form they may be.

(4) *Consumption* of organic matter. All the microbenthos respire, and except for some of the bacteria involved in the nitrogen and sulphur cycles, they derive their energy from the oxidation of organic matter, whether it be external to them or their own protoplasm.

THE TOTAL FAUNA AND FLORA PER UNIT AREA OF THE RAME HABITAT

A brief census was attempted of organisms of all sizes in a unit area of the Rame deposit, in order to place the quantity of the microbenthos in perspective with that of the larger fauna. This has only been partially achieved. The sampling has not been extensive enough to include satisfactorily the larger, scarcer members of each size group, nor has it been possible to take more than a few samples to determine the approximate abundance of the macro- and meiobenthos. Of the macrofauna only the infauna were collected.

The numbers and weights of the organisms present give a picture of the population at one time. Investigations of the type of food consumed link together the members of the community. To assess their relative importance in the turn-over of organic matter, it would be necessary to know their respiratory rates throughout the year, i.e. their rate of oxidation or consumption of organic matter. In considering the quantity of food which any one of the smaller groups may be providing for consumption by the larger fauna, the relative rates of growth and reproduction must be taken into account. This line of approach has been followed by previous ecologists, who stressed these important considerations (see Petersen, 1918; Boysen Jensen, 1919; Bornebusch, 1930; Thamdrup, 1935; and Krogh & Spärck, 1936). With the exception of Lohmann (1908, 1911) they have usually confined their attention to one or two size groups.

The Meiobenthos

Only a few core samples were examined to gain an approximate idea of the abundance of nematodes, harpactacid copepods, foraminifera, etc. Small crustaceans are known sometimes to leave the bottom and were occasionally seen to migrate up the sample tubes; the census includes all those which were taken in the sample tubes. In Table V are given the results of examination of sieved mud from two sets of samples taken (*a*) on 16 March 1939, and (*b*) on 31 May 1939.

TABLE V. NUMBERS AND DEPTH DISTRIBUTION OF MEIOBENTHOS

		Groups							
Sample	Depths in cm. below surface of core	Nematodes	Harpactacids	Ostracods	Nauplii	Cumaceans	Polychaetes	Lamellibranchs	Turbellarians
(*a*)	0·0–0·5	130	80	14	3	+	35	10	21
	0·5–1·5	100	—	—	20	—	10	—	—
	1·5–2·5	65	—	—	15	—	10	—	—
(*b*)	0·0–0·5	48	74	4	7	—	4	—	4
	0·5–1·5	36	—	—	—	—	—	—	—
	1·5–2·5	44	—	—	—	—	—	—	—

Table V shows the greatest abundance of animals to be in the surface ½ cm. layer and the restriction of adult harpactacids to that layer; nematodes and very small polychaetes were the dominant organisms deeper in the cores. Living foraminifera were entirely restricted to the extreme surface layer and were not very abundant relative to the rest of the meiobenthos (Dr E. H. Myers, personal communication).

These observations of the numbers and distribution of the small fauna are in reasonable agreement with the findings of Moore (1931) in the Clyde and Krogh & Spärck (1936) in Copenhagen Sound.

Satisfactory determination of the true live weight of these small animals is not easy. The following procedure was adopted: 50–70 copepods were placed on a cover-slip and the animals were counted while as much as possible of the extraneous water was removed with a fine pipette. They were weighed quickly; the animals were then picked off with a needle, leaving salt crystals and any debris on the cover-slip, which was then weighed again. The chief errors were due to the presence of extraneous water and to loss by evaporation from the animals themselves; these errors may be large but they oppose each other. The method was tested by weighing some larger copepods—*Tigriopus* sp.—the true density of which had previously been determined by Mr A. G. Lowndes (to whom I am much indebted for permission to use his data and material). Determinations of the density on two samples had given the average weight of an individual as 0·042 and 0·039 mg. and direct weighings gave 0·043 and 0·041 mg. The results from the two methods agreed better than was expected. The density method is only applicable when small animals can be obtained in very large numbers free from debris. A supply of copepods and nematodes was derived from Laira, where they were of similar size to those occurring at Rame. The average weight of these copepods was 0·005 mg. each and the nematodes 0·003 mg. each. From the Rame habitat, less satisfactory determinations (made on fewer animals and when evaporation from the animals was strongly suspected) gave values of 0·0024 mg. each for copepods and 0·0017 mg. each for nematodes. Turbellarians disintegrated during preparation for weighing; it was judged by inspection that they would be about the same average weight as the nematodes and that polychaetes would be appreciably heavier. The total weight of the Rame meiobenthos given in Table IX has been estimated on the basis of the individual weights of animals obtained from Laira; it is therefore an extremely rough and temporary approximation. Krogh & Spärck (1936) gave figures for the 'microfauna' from which can be calculated the average weight of copepods, 0·1–0·135 mg. each and of nematodes, 0·1 mg. each. The relative weights are similar to the Laira and Rame values, but if these organisms are the same size in the three habitats it is very tentatively suggested that perhaps there is some error in their computation, presentation of data or in the determination of the wet weight. The values for all the groups are very high, and the weight given for 'Infusoria' (0·45–1·0 mg. each) seems impossible.

For its food supply the meiobenthos is dependent on detritus, on the microbenthos and on other members of its own group. The little that is known of the food and feeding habits is tabulated below, with the sources from which the information was obtained.

NEMATODES.	Guts often empty, occasionally containing a little fine silt and possibly organic particles	Own observation
	Ingest the whole of the substrate in fresh-water muds	Baier (1935)
	Carnivores, detritus eaters and diatom eaters	Remane (1933)
OSTRACODS.	Probably mainly mud eaters	Remane (1933)

Turbellarians.	May prey on nematodes	Remane (1933)
Harpactacid copepods.	No recognizable particles in amorphous mass in hind-gut	Own observation and from Mr R. Elmhirst
	Probably scavengers and carnivores, being quite capable of chewing selected pieces of food	Mr A. G. Lowndes, personal communication
Foraminifera.	Solid particles ingested by Miliolidae	Own observation
	Some ingest solid particles, while others will grow on a diatom food supply but appear to feed by external digestion	Dr E. H. Myers and Dr M. W. Jepps, personal communications

The Macrobenthos

A brief investigation of the macrofauna of the Rame station has been made, and it may be amplified by the earlier survey of the region by Ford. The station lies nearest to his station no. 93 (see Chart to face p. 167, Ford, 1923). The community is very similar to that illustrated on p. 184 of his paper. Observations on feeding habits of macrofauna in this region were made by Hunt (1925).

Hauls were made with a Petersen grab on 12 July 1939, and the material washed through a piece of stramin netting which proved to be an effective method for the retention of the very numerous small polychaetes. Table VI

Table VI. The Macrofauna of the Rame Deposit

	No. per m.^{2}	Av. wt. of each in g.	Wt. per m.^{2} in g.	Feeding methods
Cucumaria elongata	7	5·5	38·5	S or nsD
Amphiura filiformis	15	0·07	1·05	sD
Synaptidae	4	0·3	1·2	nsD
Psammosolen chamasolen	2	5·0	10·0	S
Nephthys sp. *Glycera rouxi*	13	0·3	3·9	C
Nemertea	3	0·5	1·5	C
Sagartia sp.	2	2·0	4·0	C
Milne-Edwardsia sp.	6	2·1	12·6	C
Gonoplax rhomboides	1	2·0	2·0	C
Total of larger macrobenthos			75·0	
Lamellibranchs:				
Abra alba	222	0·003	1·2	sD
Thyasira flexuosa	104			S
others	20			S or sD
Gasteropods	42			C or sD
Polychaetes:				
Scalibregma inflata	920	0·014	31·5	nsD
Aonides oxycephala	500	0·003		sD
Magelona sp.	120	0·027		sD and C ?
Trichobranchus sp.	250	0·023		sD
other tubicolous spp.	200	0·025		sD
errant spp.	60	0·01		C
fragments	—	—		—
Amphipods	50	0·002	0·1	sD
Cumaceans	20			sD
Total of smaller macrobenthos			33·0	

Types of feeding according to Hunt's classification:
C = carnivore. sD = selective deposit feeder.
S = suspension feeder. nsD = non-selective deposit feeder.
Carnivores, 27 g. The rest, 81 g.

gives the numbers and weights per square metre. For the larger animals ten hauls, each from 0·1 m.^{2}, were examined, and for the smaller animals five of these hauls were searched. Living weights obtained by direct weighing are given. Since the average depth of the grab sample is about 10 cm. some of the rapidly burrowing crustacea will have been missed; it is known, for example, from dredge hauls that *Callianassa subterranea* is present at that station. *Echinocardium cordatum* was also found only in dredge hauls. Nomenclature is that used in the *Plymouth Marine Fauna* (1931).

The great importance of small polychaetes in this area is clearly seen from Table VI. When generation time and potential reproductive capacity are borne in mind, the polychaetes appear even more important than is immediately obvious from the weights. Details of this part of the fauna are listed in Table VII. Juvenile forms were common, but many were mature specimens of small species. The list includes several new records for the Plymouth area.

TABLE VII. POLYCHAETES FROM THE RAME DEPOSIT

(Sample collected 12 July 1939)

	No. per m.^{2}		No. per m.^{2}
Harmothoë longisetis (Grube)	r	Spionidae: indet.	r
Harmothoë lunulata (Delle Chiaje)	r	*Aonides oxycephala* (Sars)	500
Sthenelais limicola (Ehlers)	r	†*Magelona* sp.	120
Aphroditidae: indet.	r	Cirratulidae: indet.	40
Phyllodoce sp.	r	*Diplocirrus glaucus* Haase	r
Eulalia sp. possibly *E. sanguinea* (Oersted)	r	*Scalibregma inflatum* Rathke	920
Phyllodocidae: indet.	r	*Ammotrypane aulogaster* Rathke	20
Oxydromus sp.	r	*Notomastus latericus* Sars	9
Ophiodromus flexuosus Delle Chiaje	r	*Owenia fusiformis* Delle Chiaje	r
Nereis fucata (Savigny)	r	*Pectinaria* sp.	r
**Nephthys* sp. possibly *N. hystericis* McIntosh	8	*Ampharete grubei* Malmgren	r
		Amphicteis gunneri Sars	r
Nephthys sp. probably young *N. hombergi* Audouin & M. Edwards	8	Ampharetidae: indet.	r
		Melinna palmata Grube	20
Glycera rouxi Audouin & M. Edwards	10	*Terebellides stroemi* Sars	r
Goniada maculata Oersted	25	**Trichobranchus roseus* Malmgren	250
Lumbriconereis sp.	r	**Streblosoma bairdi* (Malmgren)	70
**Drilonereis filum* (Claparède)	r	Terebellidae: indet. tube only	r

r = about 1–5 per m.^{2}, i.e. 1–3 specimens taken.
* = new record for the Plymouth area.
† *Magelona* sp. has been provisionally identified as *M. cincta* (Ehlers) by Mr D. P. Wilson, who is engaged on a description of it and its larva.

I am much indebted to Mr D. P. Wilson for correcting and amplifying this list, as far as possible with limited material. Several interesting species need further investigation.

An examination of the gut contents of the bottom fauna and of the morphology of their feeding apparatus throws some light on their feeding methods and the parts of the habitat from which food was derived. Hunt's observations have largely been confirmed. A summary of the more interesting and important data is given below and the types of feeding are also indicated in Table VI.

Cucumaria elongata, whose weight in these samples comprised about a third of the total, may feed from material in suspension above the bottom but may also, according to Orton (1914), remain completely buried for long periods and presumably behave as a non-selective deposit feeder.

Scalibregma inflata is an important non-selective deposit feeder, which by means of its eversible pharynx ingests the surface or subsurface layers of the deposit. It may be of some importance in the habitat in transporting buried material to the surface. It occurred on the average at a density of one worm per 11 cm.^{2} of mud surface.

Melinna palmata, Trichobranchus roseus and *Aonides oxycephala*: the gut contents showed some selection of the finer grades of silt and occasional diatoms were found; this presumably indicates that they collect particles from the surface of the deposit by means of their ciliated tentacles.

Magelona sp. has a pair of tentacles armed with many small suckers. Besides silt, its gut contained fragments of small crustacea, indicating that the worm is in part either a carnivore or a scavenger. Larvae of *Magelona* are known to select lamellibranch larvae in the plankton (Lebour, 1922).

Nephthys spp. and *Glycera rouxi* rarely had any gut contents; when present they consisted of the remains of other polychaetes.

Gonoplax rhomboides: one specimen had been feeding on polychaetes; the gastric mill of another contained fragments of small lamellibranchs and numerous Foraminifera—*Quinquiloculina akneriana* (= *Miliolina seminulum*) and *Rotalia beccarii* had been selected; these foraminiferan species were among the commonest living on the deposit.

LAMELLIBRANCHS: as shown by Hunt, some species feed from the surface of the deposit and others from material in suspension just above the bottom, the latter habit causing a conspicuous selection of diatoms.

From Table VI it will be seen that about 25 % of the fauna is carnivorous, while the rest depends on deposited organic matter and possibly on the microorganisms associated with it. As pointed out by Hunt, the absence of recognizable remains of minute naked organisms is no proof that they are not ingested. Non-selective deposit feeders will ingest detritus and microbenthos and to some extent meiobenthos in the proportions in which they have been found in the general mass of mud. Selective deposit feeders, particularly those collecting material from the extreme surface, have access to rather more living and dead organic matter, while suspension feeders have a distinctly more nutritious food supply.

Diagrammatic Summary of the Quantities of Organisms in the Rame Deposit

The total population per unit area of the deposit is best summarized in a diagram. The diagram (Fig. 3) is intended to convey only the order of magnitude of the quantities of living matter present at one time and to illustrate a method of considering a population. It is built up from the few samples taken at various times from the Rame habitat and described in the preceding sections. It must be emphasized that the diagram is not by any means complete, first because the larger, and less frequent forms of each of the size groups have not been satisfactorily sampled, and secondly because the depth to which the samples have been taken is not sufficient to include all the organisms of each

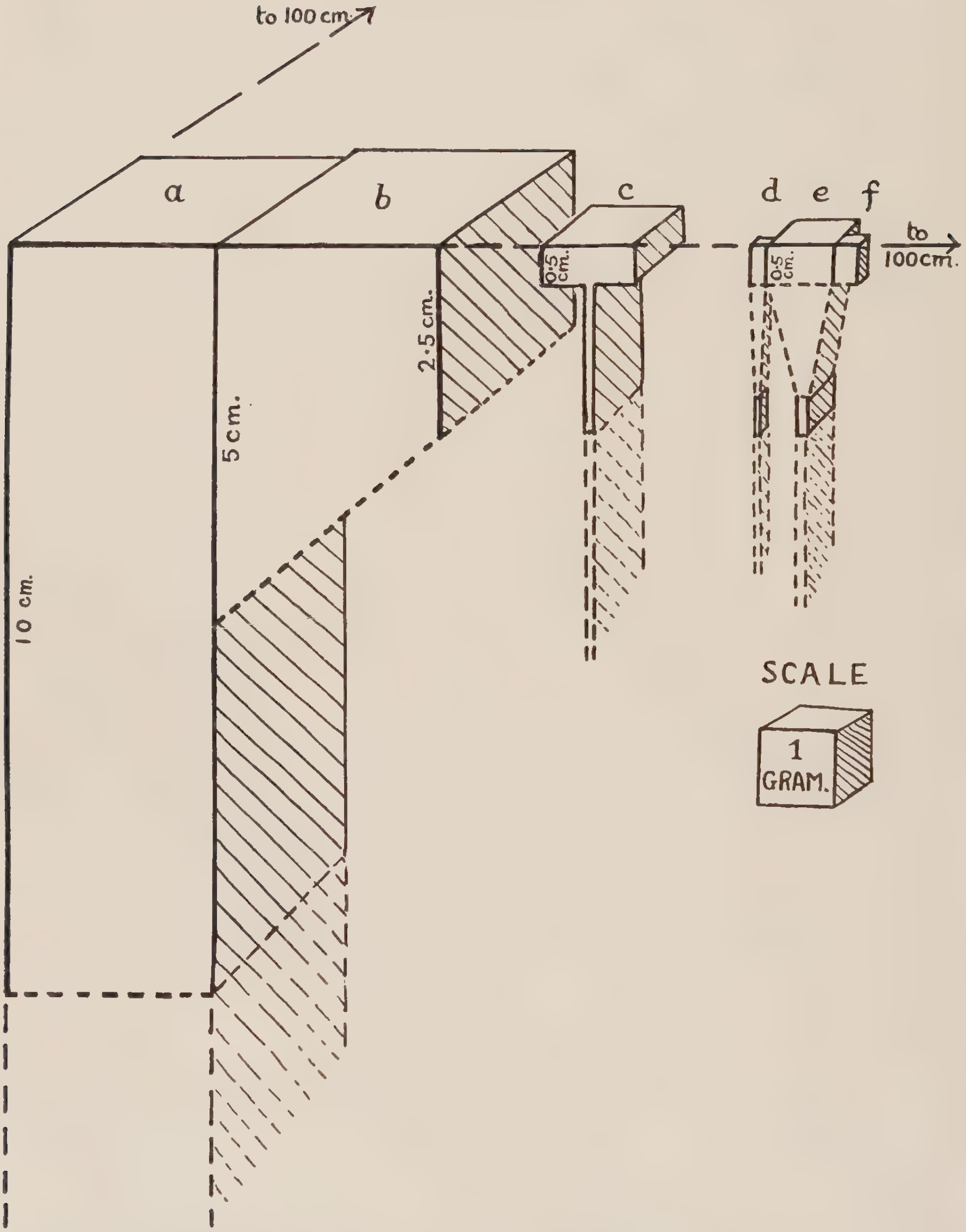
to 100 cm.
a
b
c
d
e
f
to 100 cm.
10 cm.
5 cm.
2·5 cm.
0·5 cm.
0·5 cm.
SCALE
1 GRAM.

Fig. 3. The total amount of living matter in 1 m.² of Rame mud. *a*, large macrobenthos; *b*, small macrobenthos; *c*, meiobenthos; *d–f*, microbenthos: *d*, protozoa; *e*, bacteria; *f*, bottom diatoms. For full explanation see text and Table VIII and for numerical data see Table IX.

TABLE VIII

Group	Method of collection and analysis	Range of individual weight	Date of collection
Macrobenthos (*a*)	Peterson grab, average depth of sample 10 cm.	Over 100 mg. (up to 6 g.)	12. vii. 39
Macrobenthos (*b*)	Same sample, sieve 1 × 1 mm.	100–1 mg.	12. vii. 39
Meiobenthos (*c*)	Core sampler, sieve 0·1 × 0·1 mm.	0·05–0·0001 mg. usually to 0·001 mg.	16. iii. 39 31. v. 39
Microbenthos (*d–f*)	Core sampler, culture	1 × 10^{-4} to 1 × 10^{-9} mg.	27. iv. 39

TABLE IX

	Weight per m.^{2} in g.	No. of individuals per m.^{2}
LARGE MACROBENTHOS (*a*):	75	56
Cucumaria elongata, dominant by wt.		
Some burrowing crustacea missed below 10 cm.	+	+
SMALL MACROBENTHOS (*b*):	33	2,300
Small polychaetes dominant, probably restricted to top layer, surface to 2·5–5 cm.		
Some small polychaetes missed	+	+
MEIOBENTHOS (*c*):		
Numbers minimal; weights of some groups uncertain; total weight a very rough approximation.		
Variety of species—in surface ½ cm.	0·8	86,800
Nematodes and a few polychaetes, 0·5–2·5 cm.	0·35	60,000
Nematodes probably extending deeper	+	+
Foraminifera, a few living ones confined to surface ½ cm.	+	+
MICROBENTHOS:		
Large ciliates: some probably occurred, too scarce to be counted in culture	+	+
PROTOZOA (*d*):		
Minimal numbers, trophic and resting forms:		
In surface ½ cm.	0·02	283,000,000
At 2·5–3·0 cm.	0·01	15,000,000
BACTERIA (*e*):		
Plate count × 100; a very rough approximation to total live count: In surface ½ cm.	0·36	355,000,000,000
At 2·5–3·0 cm.	0·07	72,000,000,000
BOTTOM DIATOMS (*f*):		
Minimal numbers; only those on surface and probably capable of photosynthesizing are shown in diagram:		
In surface ½ cm.	0·05	590,000,000
At 2·5–3·0 cm.	0·01	140,000,000

PLANKTONIC DIATOMS, obtained by the culture method, represent an unknown fraction of the total diatoms deposited, either as resting spores or in a more or less moribund condition. The culture method showed 1·64 g. per m.^{2} to be present in the surface layer on 27 April and 0·04 g. in the layer at 2·5–3·0 cm.; the highest figures obtained were about 25 g. of living planktonic diatoms per square metre down to a depth of ½ cm. The planktonic diatoms, with the less recognizable detritus are best regarded as the organic matter deposited on to the habitat from an external source, and not being indigenous members of the benthos they have not been included in the diagram.

group. These omissions are indicated in the diagram. Ideal sampling would give the numbers and weights of animals per unit area, e.g. per square metre, of deposit to the maximum depth of occurrence of the fauna, all samples being collected on the same date. The vertical distribution is indicated on the vertical faces of the rectangular blocks, the presentation being somewhat influenced by the methods of sampling. The square horizontal areas are adjusted to give volumes representing the total live weights of organisms in the different groups. The density is conventionally taken as 1, i.e. 1 c.c., is equivalent to 1 g. The blocks are represented in perspective, the depth and width of the front face being absolute measurements; the blocks are placed in one corner of the whole square metre. Epifauna is excluded. Data concerning the method of collection and subdivision into size groups are given in Table VIII and the actual figures from which Fig. 3 is built up are given in Table IX.

The Proportion of Organic Matter which consists of living Organisms

The total organic matter is about 1 %, i.e. 10 mg. per g. dry weight of the deposit at Rame. It is obvious that the largest animals would not be included when making chemical determinations, and the chance of encountering them is very small. The micro- and meiobenthos and possibly some of the small macrobenthos would, however, be included in such determinations. The present state of our knowledge of the components of the organic carbon in a deposit is summarized below. 'Maximal' values (see p. 534) for the microbenthos and diatoms are used. Table X gives an indication of the composition of the organic matter ingested by a non-selective deposit feeder:

TABLE X

		Proportion of the total organic matter
In the surface ½ cm.	True microbenthos	0·3–0·03 %
	Planktonic diatoms	At least 2·5–0·015 %
Deeper in the deposit	True microbenthos	1/5th to 1/10th of the above
	Planktonic diatoms	A smaller proportion
In the top 2·5 cm.	Meiobenthos	At least 0·02 %
Fraction biologically available to bacteria (see Waksman & Hotchkiss, 1938; and Anderson, 1939)		10–17 %
Resistant fraction, mostly finally buried		The rest

There is of course far more dead organic matter in the deposit than total organisms. The total indigenous fauna and flora amount to about 5–10 g. of organic carbon per square metre down to a depth of 10 cm., while the detritus amounts to about 1 kg. of organic carbon per square metre down to a similar depth. Of this, as seen in Table X, only a very small proportion consists of recently deposited or readily available food.

DISCUSSION

In assessing the relative importance of the different groups in the turn-over of organic matter, the determination of the numbers and weights of organisms of all sizes is only the first preliminary. The relative rates of respiration must be taken into account. Krogh & Spärck (1936) suggested that the small animals, such as copepods and nematodes, use three or four times as much oxygen per gram of living tissue per hour as do members of the macrofauna, and it is well known that bacteria and protozoa have a much higher rate of respiration than metazoa (see Krogh, 1916; Heilbrunn, 1937, pp. 197–201; and Cook & Haldane, 1931). The smaller organisms therefore utilize relatively much more of the food supplies in the habitat than would appear from a direct comparison of quantities such as is made in Fig. 3.

It is impossible at present to do more than point out this important aspect of the subject because of the scarcity of data on the respiratory rates of the organisms concerned, and the complete lack of determinations under conditions of oxygen concentration, population density, food supply and temperature comparable to those found in the mud habitat. Some of the macrofauna may have an important mechanical effect on oxidation of organic matter in the deposit. For example, non-selective deposit feeders such as *Scalibregma inflata*, and forms such as tubicolous polychaetes and *Calianassa subterranea* which maintain currents of water in their tubes or burrows, may facilitate direct chemical oxidation or prolong the activities of aerobic bacteria.

The problem of the role of living micro-organisms in the food of detritus eaters is not yet capable of solution, because neither the food requirements nor the rate of feeding of any member of the Rame benthos have yet been worked out. It is to be expected that the quantitative food requirements and utilization will vary greatly between different species and with different modes of feeding. In so far as it throws any light on the subject, work (in progress) on *Arenicola marina*, a non-selective deposit feeder from the littoral zone, may be mentioned. From a study of this animal it seems that only a very small amount of food is required for maintenance (estimated from its respiratory rate), but that the quantity of micro-organisms ingested in a sand deposit is insufficient to meet this requirement. Account must be taken of the quantity of living food in a mouthful of ingested deposit, of the rate of feeding, of the population density of detritus eaters and hence of the interval which elapses before the same mud is again ingested. On the Rame ground it seems probable that, at the time of heavy deposition of planktonic diatoms, the quantity of diatoms present in the surface layers would be adequate for an animal feeding non-selectively and having a rate of feeding and food requirements of the same order as those of *Arenicola*. The quantity of true microbenthos and of planktonic diatoms found at other times of the year is such as to suggest that a non-selective deposit feeder would be largely dependent on dead detritic matter to form the bulk of its food supply. Selective deposit feeders and suspension feeders have access to a richer food supply in the extreme surface layer.

Although quantitatively inadequate to be of appreciable calorific value the microbenthos or the freshly deposited phytoplankton may be essential components in the food of the macrofauna in that they contain vitamins in all probability absent from detritus. This suggestion was first made by Hunt

(1925), but in the light of more recent work on vitamins (see also Fox, 1937) it requires modification in detail.

The food value of a population depends on the quantity present at any one time and the rate at which depletions are made good, i.e. the rate at which mud, in the form of faecal pellets, becomes part of the general habitat again and is reinvaded by such types of micro-organisms as are destroyed by passage through the gut of a metazoan. The rate of reproduction of the micro-organisms must also be sufficient for the invasion of newly deposited silt and detritus, if the community is to remain balanced. For bacteria and protozoa the food concentration, both local and general, plays so important a part in determining the rate of reproduction that it would be very difficult to find the normal rate in the habitat. From laboratory observations and literature (see Cutler & Crump, 1924; Baier, 1935; and Mare, unpublished thesis) it can be tentatively suggested that the generation time for bacteria is about 1–3 hr., for small flagellates about 10 hr. or less, for small ciliates 10–20 hr., and for bottom diatoms possibly about 2–3 days, under the conditions of light, temperature and food concentration in the Rame habitat. These rates, coupled with the quantities listed in Tables I–IV, make it probable that the reproductive rate would not be a limiting factor in the population density of the microbenthos providing that there were ample organic matter available. ZoBell & Feltham (1938) estimated that 'several milligrams of bacterial substance are synthesized per day in the top 5 cm. of 1 sq. metre of marine muds and although the majority is consumed by bottom animals the diet is perhaps supplemented by other types of food'.[1] It was shown in Table VI that 75 % of the Rame macrofauna was dependent on detritus and micro-organisms in and immediately above the habitat. The meiobenthos consists of both potential food supply and consumers. In comparing the annual potential food supply with the quantity of consumers, allowance must be made for the number of generations per annum of consumers, their food requirements for energy production and the extent to which the young stages draw on the food supply but fail to reach maturity. These factors are at present completely unknown.

The food relationships within the benthic community cannot be fully elucidated without reference to the relationship between the benthos and the water above the habitat, see Fig. 1. This subject requires an immense amount of knowledge accumulated from the work of many investigators, and the work already done could only be dealt with adequately in a separate review. Studies on these lines are much less advanced in the sea than in freshwater lakes, where the greater accumulation of data and the circumscribed water mass make it possible to estimate the total productivity of the water and the part utilized by the bottom fauna (see Rawson, 1930; Juday, 1940; and Deevey, 1941).

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[1] The same authors (1942) investigated a very abundant bacterial flora, important in various ways, in a Californian tidal mud flat. Taking 10 million as the average number of living bacteria per c.c. of mud, they estimated that about 11 g. dry wt. of bacterial substance would be synthesized per day per cubic foot of mud, thus forming an important source of food in the habitat. It should be noted that bacteria are far more numerous in that intertidal habitat than in the sublittoral Rame ground and that probably the fauna is also more abundant.

An outline of the balance-sheet of events on the sea bottom is given here, so that the small additions made in the present work may be seen in their correct places in the whole cycle. For an understanding of the annual cycle on the sea bottom, quantitative knowledge on the following subjects is required:

The annual deposition from the water, of plant, animal and faecal material.

The amount of photosynthetic production (if any) in the habitat itself (see p. 535).

The conversion of part of the detritus into protoplasm of micro-organisms and the mineralization of part. The numbers and quantities (see pp. 530–533) and the reproductive and respiratory rates when fully known would enable the annual production of living matter and the amount of turn-over of organic matter for which these organisms are responsible to be assessed.

The consumption of some of the living organisms and of detritus in oxidative metabolism by detritus eaters of all sizes. The numbers and weights (see pp 539–545) and the annual food requirements for energy production need to be known.

The accumulation of organic matter in the growth of the larger fauna, including the rate and efficiency of growth.

The utilization of detritus eaters by the carnivorous members of the community, as material for oxidation and growth.

The liberation of reproductive products to the water and the return of larvae to the habitat.

The removal of food from the habitat and of members of the community by scavengers and by predators from outside the habitat, e.g. by bottom-feeding fish. If the community remains approximately constant in total weight, the quantity removed is equal to the sum of the annual growth increments of such members of the fauna as are of a nature and size to serve as suitable food.

The return of nutrient salts and other important substances into the water as the result of bacterial activity and as excretory products of the fauna.

The loss from the food cycle of matter finally buried. This will consist of that resistant fraction which is biologically unavailable to bacteria of the upper layer and indigestible to the fauna. There may be some anaerobic decomposition of this material by bacteria deep in the deposit and perhaps slow autolysis; some marine humus may thus eventually be converted into petroleum.

Summary

An attempt has been made to deal briefly but quantitatively with all size groups of the fauna and flora in a marine mud deposit.

It has been necessary to propose the following new terminology: the *Macrobenthos*, which is here equivalent to the macrofauna, the *Meiobenthos*, under which term are included copepods, nematodes, foraminifera, etc., and the *Microbenthos*, comprising the rest of the protozoa, bacteria, bottom diatoms and other algae; planktonic diatoms and coloured flagellates also occur on the bottom in the region investigated but are not regarded as true microbenthos.

A quantitative ecological study of the microbenthos has been started and the habitat is first described, stress being laid on points of importance to the micro-organisms.

The census method for bacteria was an agar-plate method and that for the protozoa and diatoms was a dilution culture method modified from that used by soil microbiologists.

These methods give minimal values for the total population and may safely be used for comparative purposes. The results are still tentative.

Typical figures for the minimal numbers and volumes of living protoplasm per gram of dry mud in the top $\frac{1}{2}$ cm. layer are given in Tables I–IV.

The surface layer is much richer in all types of organisms than is the mud deeper in the cores. There is considerable local horizontal variation in numbers of bacteria and protozoa, suggesting dense aggregations.

Diatoms contribute by far the greatest bulk to the total volume of living micro-organisms; planktonic diatoms, particularly during the summer, completely outweigh the true microbenthos.

A seasonal variation has been shown in the numbers of bottom and planktonic diatoms. There was a fourfold increase in the quantity of bottom diatoms from winter to summer and while in the winter they were nearly as important as with the planktonic species (the ratio by volume being 1 : 1·7), the planktonic species became overwhelmingly important in the summer (the ratio becoming 1 : 67).

The distribution of bottom diatoms and other photosynthetic organisms in the littoral and sublittoral zones has been briefly investigated.

At the Rame mud station there are many more organisms per gram of total deposit than there are in the Eddystone shell gravel. The proportion of living matter in the fine fraction of the gravel is, however, much higher and of the same order as at Rame.

Food relationships in the microbenthos are suggested and it is pointed out that the protozoa are sufficiently numerous for their interaction with the bacterial population to be highly important.

The total volume of the true microbenthos present at any one time is extremely small. Even in the surface layers at the Rame station its carbon content was only about 0·3–0·03 % of the total organic carbon in the deposit. planktonic diatoms formed at least 2·5 % of the total organic carbon in the spring and early summer.

The meio- and macrobenthos were briefly investigated, the numbers and weights per m.^{2} being determined.

An interesting fauna of small polychaetes includes several new records for the Plymouth area.

The majority of the fauna ingest detritus either from the surface layer of the deposit or from the water immediately above the bottom.

The numbers and weights of most of the fauna and flora belonging to all size groups in the one habitat, as far as they were determined, are shown in Table IX and diagrammatically in Fig. 3. The relative amount of the living to the total organic matter present is given in Table X. These facts, when more adequately known and when combined with further qualitative observations on the food and feeding methods, will form a necessary basis for the discussion of food relationships throughout the community. Taking into account the amount of microbenthos represented in Fig. 3 and its respiratory and reproductive rates, the micro-organisms are probably capable of playing a not inconsiderable part in the turn-over of organic matter in the habitat when compared with the larger fauna.

The information so far accumulated is of a preliminary nature, and the numerical data presented must always be taken as indicating the magnitude of the quantities involved and not their exact values. Differences are so great, however, that it is possible to draw tentative conclusions. As it is no longer possible to obtain samples from the original habitat, these conclusions are put forward now, subject perhaps to modification in the future.

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THE DISPLACEMENT METHOD OF WEIGHING LIVING AQUATIC ORGANISMS

By A. G. Lowndes

(Text-fig. 1)

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INTRODUCTION

The work to be described constitutes the first successful attempt to weigh a living aquatic organism such as a fish, a prawn or an embryo without removing it from its aquatic environment. My primary object has been to find the *density of living aquatic organisms*, and this has been done throughout; but since it is essential to *weigh* before any form of quantitative analysis can be made, it would appear that this is the more basic problem. The determination of the *density* has as its final goal the problem of the distribution and the amount of energy required for that distribution, while the technique of weighing a living aquatic organism is fundamental in biochemistry and embryology and many other branches of biology.

The work was first started in 1936, but it was not till 1938 that I was able to give my whole time to it at the Plymouth Laboratory. For this work I received a generous Government grant through the Royal Society, together with a similar grant from the British Association, and I am indebted to the Marine Biological Association for laboratory accommodation and all possible facilities. To all these bodies I wish to express my gratitude.

The war has brought many and serious interruptions to the work, and though in normal circumstances publication at this stage might well be thought premature, it has been thought best in existing conditions to give an account of the results so far obtained.

METHODS

To find the density of an organism it is necessary to weigh it and to find its volume, and since many people have suggested that this can be done directly it is thought advisable to discuss the method briefly. A large prawn was known to weigh 5·253 g. with an error of less than ± 0·007 g. It was weighed directly by placing it in a dry stoppered weighing tube and weighing the whole. Then the prawn was removed and the tube again weighed. The weight was found to be 5·663 g., indicating an error of 8 %. The volume of the same prawn was known to be 4·80 ml. with an error of rather less than ± 0·010 ml. Its volume was obtained directly by taking two 100 ml. graduated cylinders and rinsing them both with sea water. One was filled with sea water, the prawn inserted and the water levelled to the 100 ml. mark. The water was then poured into the other cylinder leaving the prawn behind, and it was drained for 15 sec. The volume of the prawn thus obtained was 6·0 ml., thus indicating an error of 24 %. In nearly all the results obtained by the displacement method (to be described later) the weight was also found by the direct method and the percentage error given. Direct weighing forms a useful check, but for accurate work it is useless.

It has also been suggested that a fish can be weighed by weighing a tank of water and dropping the fish into it, but apart from the fact that it is not possible to dry the fish, or even to drain it properly without bringing about serious physiological changes, there is the difficulty of weighing a tank of water. The accuracy with which anything can be weighed depends primarily on the earth's gravitational constant, and it can be shown on theoretical grounds that the extreme limit is 1 part per 1,000,000. Thus it is not possible to weigh a kilogram to the fourth decimal place of a gram. In practice the seventh figure in any single weighing is meaningless. If a tank holding 10 l. of water is taken, and this amount is certainly not sufficient to cover a large dogfish, a lobster or a crab, a weight of nearly 20 kg. has to be dealt with, and since the displacement value of the majority of organisms in sea water is quite small, the difference in weight cannot be measured by direct weighing with reasonable success. Similarly, since a measuring cylinder always carries 100 divisions unless it is specially made, a litre cylinder having a diameter of about 3 in. will only read to 10 ml. A measuring cylinder which would take a large crab some 10 in. in length of carapace could only be read to about 100 ml. The above difficulties should be sufficient to indicate that a special technique is essential.

The Displacement Method

The general principle of the method I have adopted lies in the use of the specific gravity or density bottle, which is probably the most accurate of all volumetric apparatus. The bottle is weighed full, the animal inserted and the bottle again weighed. There is almost without exception an increase in weight, showing that all marine organisms have a density greater than that of sea water. The method can, however, only be rendered strictly quantitative if it is possible to obtain the volume of the organism and the density of the sea water together with the volume of the density bottle. The last two are easily obtained by standard methods and present no great difficulty. The important point is the accurate determination of the volume of the organism.

Volume Determination. If a density bottle is filled with sea water and the contents poured into excess of silver nitrate, a definite weight of silver chloride or rather silver halide is obtained. This precipitate can be weighed, and one is actually using what is probably the most accurate of all gravimetric determinations in standard analytical chemistry. If now the same bottle is again filled with sea water, a shrimp inserted, and the water again poured into excess of silver nitrate, a smaller weight of silver halide will be obtained. From these two weights it is possible to obtain the volume of the shrimp with extreme accuracy; but there is, however, the rinsing of the shrimp to be taken into account. In the first instance the bottle was rinsed with distilled water; but this cannot be used for rinsing the shrimp because of the difference in osmotic pressure between it and sea water. The shrimp must therefore be rinsed with a solution devoid of halide but having the same osmotic pressure as sea water. The obvious washing or rinsing solution is one of sodium nitrate to which a little calcium nitrate is added. In practice the shrimp is retained on a filter and rinsed quickly with the isotonic solution. The volume of the shrimp having been determined, its weight and density can be obtained and the shrimp can go back to the aquarium for further experimental work. An animal tissue would naturally be weighed in a suitable Ringer solution, otherwise the procedure is identical.

In actual practice the technique is rather laborious and less simple than it sounds at first. It consists, however, in the preparation of the isotonic solution of sodium nitrate and the making and standardization of suitable density bottles. Dealing with the solution first: 4 lb. of pure sodium nitrate are dissolved in about 8 l. of water. 100 ml. of pure nitric acid are neutralized with excess of calcium carbonate and the solution boiled and filtered into the sodium nitrate solution. The pH is brought up to that of sea water by adding a dilute solution of sodium carbonate. In practice this neutralization is quite easy, for on adding the sodium carbonate a precipitate is formed which dissolves less and less readily as one approaches the end-point. The freezing-point of the solution is determined accurately with the Beckman apparatus at different concentrations and a convenient table constructed. It is found that if 350 ml.

of the above solution are made up to a litre, a solution very slightly hypertonic to the Plymouth circulation sea water is obtained.

In dealing with organisms living in brackish water the freezing-point of the environment must first be ascertained and then the corresponding solution of nitrate made up. The solution should be as near isotonic with that of the environment as possible, but it should be hypertonic rather than hypotonic, for if the nitrate solution is hypotonic the organism will take in water and give out chlorides, while if it is hypertonic it will give out water, which will make no difference to the measurement of the volume at this stage, and the rate at which the organism will absorb chlorides will be so slow as to be negligible, apart from the fact that there is very little chloride available for it to absorb. It should also be remembered that the nitrate solution need only be in contact with the organism for a very short time, usually a few seconds or half a minute at the most. The bivalent calcium ion has the effect of slowing down the rate of exchange of ions considerably.

That this nitrate solution has no detrimental effect on the organisms is shown by the fact that they will survive being placed in it many times in succession. Thus the same specimen of the first larva of a lobster has been rinsed thoroughly in the solution six times during the one day. The same is true for *Anemonia sulcata* and for the sponge *Ficulina ficus*. If a young nursehound is removed from sea water, it soon forms within the pharynx quantities of mucus, but nothing of the kind appears when it is immersed in the nitrate solution.

Density Bottles. The greatest difficulties in the displacement method are those afforded by the density bottles. While it is easy to clean and dry a crucible and handle it with forceps, and finally leave it on the balance for some time before weighing, such things cannot be done with a density bottle containing a liquid, and the difficulty becomes far greater if the bottle contains a living organism which is to survive. The accuracy of the method is determined by the consistency of consecutive readings with a bottle which is filled, dried on the outside and placed on the balance. If the difference does not lie within a milligram it is clearly useless to weigh to the fourth decimal place. The difficulty has been long recognized and partly overcome by such devices as the Bousfield and Sprengel pycnometers; but these are useless for weighing anything but the very smallest organisms by the displacement method. If a 100 ml. density bottle when full of sea water can be weighed accurately to within a milligram the percentage error is small; but we are concerned with the displacement weight, or the difference between the bottle full and the bottle with the organism inserted, and since this seldom amounts to more than a few grams the percentage error may be considerable.

The density bottles used range from those holding 0·5 ml. to those holding nearly 8 l. The smaller bottles, which are always of the type shown in the diagram (Fig. 1*a*), are always placed for weighing in a close-fitting outer covering (Fig. 1*b*). The water evaporating from the bottle itself quickly saturates the relatively small space enclosed, while the rate of diffusion between the air in the outer case and the desiccated atmosphere of the balance itself is extremely slow. A density bottle of this type, placed in its outer case, showed no increase or decrease in weight at the end of half an hour on the most sensitive balance used. The outer case need never be removed from the balance case. Usually a reasonably accurate weighing can be made with one of these smaller bottles in 30 sec. at the most. The balances used with bottles up to 100 ml. are the air-damped Sartorious type or the new Oertling aperiodic type, or in the smallest bottles a Sartorious air-damped microbalance, these balances carrying a maximum load of 200, 100 and 20 g. respectively with corresponding sensitivities.

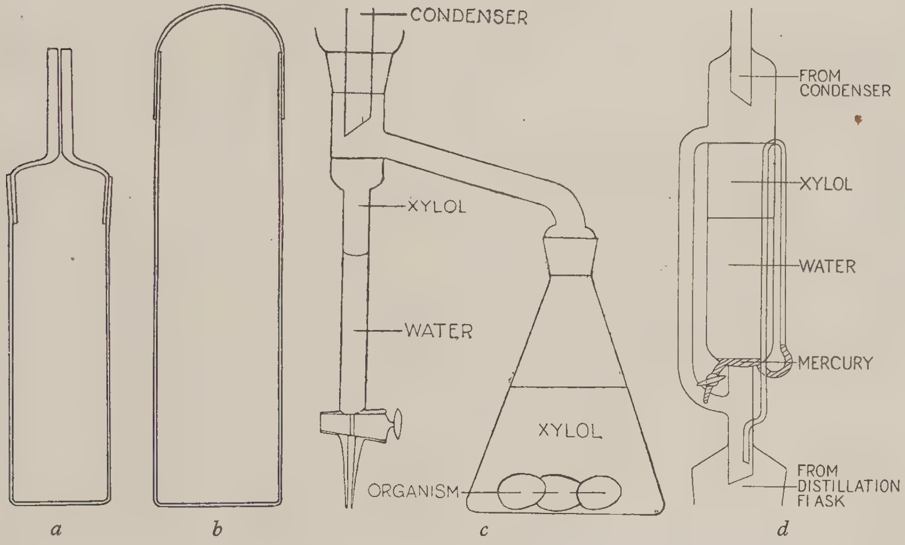

Fig. 1. *a*, type of density bottle used. *b*, outer case for taking the density bottle while being weighed. *c*, an ordinary Dean & Stark's apparatus used for the estimation of water. *d*, an ordinary Soxhlet Extractor used as a Dean & Stark's tube. The siphon tube is blocked with a little mercury. Water collects in the extractor and xylol automatically returns to the distillation flask.

For the weighing of prawns a special density bottle was made. Some long thin weighing bottles were found in the laboratory. They were just over 4 in. long and fitted with a good ground-glass stopper. A small V notch was made in the stopper with a file and the bottle carefully standardized by use of N.P.L. tables. It was easy to make up a closely fitting outer case. The volume of the bottle was about 35 ml., and since 1 ml. of sea water gives about 0·08 g. of silver halide precipitate, the whole contents of the bottle gave about 2·8 g. of the silver salt. This amount of silver salt is easily handled with modern sinter glass crucibles and can be weighed accurately. The limit of accuracy here is clearly the accuracy with which the bottle can be dried and weighed consistently. The only real test is the error obtained by taking the same prawn, weighing it several times and comparing the result; but unfortunately this is not so simple as it sounds. No living aquatic animal is unaffected by handling, and in prawns scales or spines may be lost or the animal may excrete or feed during the process. Moreover, the aerated sea water frequently gives off bubbles of air and these may seriously affect the result. It is true that the air could be expelled from the sea water by first boiling it, but the animals are not living in freshly boiled sea water.

The following table shows the extremes obtained by weighing the same prawn four times by the displacement method and thus obtaining the extremes of weight, volume, density and sinking factor (the last-named is described later). It will be seen that the figures for the last two agree well to within 1 part per 10,000, and this appears to be about the limit of accuracy for a very favourable organism.

Volume ml.	Weight g.	Density 15·2° C.	Sinking factor	% error in volume
4·810	5·2597	1·0935	1066·2	0·026
4·795	5·2438	1·0936	1066·3	0·026

While the above measurements were being taken the prawn in question shed one of its uropods; this accounts for the difference in weight and volume, but as might be expected it has not seriously affected the density or sinking factor.

Apart from the expense, it is not feasible to work with a much greater weight of silver halide than that used in this experiment; if a larger density bottle is used, its contents are transferred to a standard flask, a suitable volume extracted with a pipette and the silver halide estimated gravimetrically, or in certain cases by titration. The limiting factor in this method is the accuracy of the pipette, which increases the error considerably, and if one resorts to a titration the percentage error may be considerable. The same prawn was again weighed by the displacement method, but a titration was substituted for a gravimetric estimation. This gave the following result:

Volume ml.	Weight g.	Density 15·2° C.	Sinking factor	% error in volume
4·717	5·1664	1·095	1068·0	0·77

From the above figures it will be seen that a titration is scarcely satisfactory, but much depends on what proportion of the volume of the density bottle is occupied by the organism. An example will make this clear. A full-sized prawn in berry will just go into an ordinary $\frac{3}{4}$ in. test-tube and occupy about three-quarters of the length. The volume of the test-tube level with the tip of the rostrum of the prawn is about 35 ml., but the volume of the prawn itself is only 5 ml. If the sea water from the test-tube is poured into a 250 ml. flask, in the one case 35 ml. of sea water will be added and in the other 30 ml., and the difference in these two solutions is too small to be estimated with any great degree of accuracy. The actual figures approximate to the following: If a 35 ml. density bottle full of sea water is taken and rinsed to fill a 250 ml. flask, then 25 ml. of this solution will be titrated by 19·6 ml. of $N/10$ silver nitrate. If the prawn, of an assumed volume of 5 ml., is inserted into the density bottle, which is again washed out into the same 250 ml. flask, 25 ml. of this solution will be titrated by 16·8 ml. of the silver nitrate. Assuming the accuracy of the titration to be within ±0·02 ml. the percentage error is $\frac{0·02 \times 100}{19·6 - 16·8} = 0·71$, and to this must be added a further error of 0·02 due to dilution. If, on the other hand, the solutions are estimated gravimetrically, the figures are as follows: 35 ml. of sea water will give 2·8 g. of silver halide while 30 ml. will give 2·4 g. These weights can be measured accurately and in practice they will give the fourth place of decimals. The percentage error is therefore $\frac{0·0001 \times 100}{2·8 - 2·4} = 0·025$, and since there is no dilution this will be the total percentage error. Thus gravimetrically the percentage error is 0·025 compared with an error of 0·73 by titration. In practice I have almost entirely discarded titrations in this work.

It might be expected that an even greater degree of accuracy could be obtained as the result of this gravimetric work, but in general this cannot be done. Greater accuracy can only be obtained with such material as the early larvae of crabs or lobsters which can be packed into a density bottle very closely and made to occupy a large proportion of its volume. It must be remembered that the density bottle is used as a piece of volumetric apparatus, and this must put its own limit on the work. A density bottle cannot be graduated to the fifth decimal place of 1 ml., and it is being used with organisms and not dry chemicals. It should also be borne in mind that sea water itself is not a pure solution of sodium chloride.

For bottles holding over 100 ml. a special technique is required and practice has shown that excellent density bottles are easily made by taking good wide-mouthed bottles with suitable stoppers and cutting a V notch in the stopper. Excellent bottles for this purpose are made in the following sizes: 8, 12, 16, 32, and 64 oz. The 8 oz. bottle has an approximate volume of 315 ml. and the 64 oz. holds nearly 2 l. The stopper must be marked so that it can be replaced in exactly the same position each time, and the bottles must be accurately standardized by means of N.P.L. tables. Evaporation from these wide-mouthed bottles is a difficulty, but for reasons which need not be discussed it is far less in these bottles with a V notch than it is in those bottles with the ordinary perforated stoppers. For weighing with these larger bottles I have used two Oertling balances, the one taking a maximum load of 2 kg. and the other 5 kg. Weighing is here a much slower process, but since the animals are larger and more robust they can remain in one of these bottles for a longer time without suffering any apparent injury. With care the same animal can nearly always be weighed twice. The first time an approximate weighing is obtained; the animal is then returned to circulation for a short time, and on the second weighing an accurate figure is quickly obtained. If necessary, evaporation can be overcome completely by placing the large bottles in an outer covering as recommended for the smaller bottles; well-made tins can be used. The disadvantage with the larger bottles is that the contents must be poured into a standard flask and an aliquot part extracted with a pipette, and this naturally limits the accuracy. In practice 50 ml. are extracted with the pipette and the silver halide estimated gravimetrically. These larger bottles are admirable for weighing nearly full-sized dogfish.

The work has now been extended so that the largest crabs and lobsters can be weighed, but this has involved a rather special technique. The density bottle presented considerable difficulty, but I was fortunate enough to obtain a large vessel with a really well-fitting stopper, made for the storage of sterilized instruments and dressings. By cutting two V notches in the stopper it was possible to convert it into a good density bottle. It held over $7\frac{1}{2}$ l. and had a mouth about 8 in. across. Two flasks of suitable size had to be obtained, and this presented some difficulty for they had to hold at least 10 l. The neck of one of them was carefully marked. It was unnecessary to standardize this flask provided there was some means of filling it up to the mark consistently. Finally, a suitable balance had to be obtained, and this was eventually supplied by Messrs Avery and Co., Ltd. It is capable of taking a load of well over 20 kg. and can be read to the nearest gram. The balance has the great advantage of being damped by a special kind of oil-bath and weighings are thus instantaneous.

With practice the jar could be filled and the weight read consistently to within a gram, and since the whole weighed just over 10 kg. the error was not more than 1 in 10,000. This apparatus was only used for the largest animals, and provided the displacement value was over 100 g. the percentage error in weight would be less than 1. Since any error in weight affects the weight and volume of the animal in question in the same direction and approximately to the same extent (1·025 : 1·0), the density and the sinking factor can be relied upon at least to the fourth digit. Titration would introduce far too great an error to be of any service.

The Limits of the Method. As an upper limit it may be stated that the largest lobsters to be obtained from the ordinary market and almost the largest crabs can be dealt with by the method outlined above. Nursehounds up to a metre long and even still longer congers can also be weighed, but the handling of these animals is difficult. The chief danger is that the large crabs or lobsters may break the jar with their claws. It is, however, the lower limit that is the most important, and in this respect the method is disappointing. The smallest density bottle used holds 0·5 ml., and so far I have found it impossible to place it consistently on the balance when full, with an error of less than 0·1 mg. This is about ten times the displacement value given by ten specimens of *Calanus*, and it is therefore impossible to weigh a single specimen of the copepod. It is possible, however, to obtain a reasonably accurate figure by placing 100 specimens in the bottle. Similarly, it has not been found possible to weigh satisfactorily a single specimen of the first larva of the lobster. On the other hand, it is quite easy to hatch out the first larvae of a single prawn in berry and get a very accurate estimate of the density by weighing several hundreds of them at once.

Density. The Units Used

By density is meant the mass of unit volume, and the unit of volume is the millilitre and not the cubic centimetre. In all cases the densities have been calculated according to the formula

$$d_{4^\circ}^{t^\circ}=\frac{W'D}{W}-\frac{0{\cdot}0012\,(W'-W)}{W}.$$

$d_{4^\circ}^{t^\circ}$ means the density of the liquid at t° C. compared with that of distilled water at 4° C. (its maximum density). W is the weight of distilled water at t° C. and W' is the weight of sea water at the same temperature. The second part of the formula is the correction for the buoyancy of air. The formula is that given in *Practical Physical Chemistry* by Alexander Findlay (Longmans, London). The density of water at different temperatures is that given on p. 64 of the same book. Owing to the discovery of 'heavy water' the whole concept of pure distilled water has been changed. The distilled water used for standardization of apparatus in this work was obtained by distilling the Plymouth tap water first in an 'Atmos Still' and then redistilling it in a special still of Monax glass.

The Sinking Factor

Little information is conveyed by a determination of the density of the organism unless the density of the environment is determined at the same instant and under the same conditions of temperature. It is usually assumed that the density of sea water is 1·03, but this is scarcely correct for Plymouth sea water, and naturally the density varies with the temperature. What is required is a simple expression which will at once show the relation of the organism to its environment at the time of the determination. As an example: it was found that the density of a certain dogfish at a temperature of 17·2° C. was 1·0761, while the density of the environment at the same temperature was 1·02552. The obvious way of comparing these two densities is by means of the ratio $\frac{\text{Density of dogfish}}{\text{Density of the sea water}}$ or 1·0761/1·02552, which gives 1·0493. This, however, is not an easy number to bear in mind, and it is therefore multiplied by 1000 and the last figure discarded. The sinking factor of the dogfish in question would thus be 1049. A few examples of other results may be given. Only two determinations were made with the conger, and in both it was impossible to get a displacement value at all; in other words, the density of the fish was that of its environment, and the sinking factor (or s.f.) is therefore 1000. In the common stickleback the factor was found to be 1002, in the dogfish 1049, while in lobsters a sinking factor of 1140 has been obtained. This evidently means that a conger need make no effort to keep itself off the bottom; a stickleback requires a little more effort, while a dogfish must exert a much greater effort. Lobsters require a great expenditure of energy to raise themselves off the bottom.

THE PERCENTAGE OF WATER

Previously this was obtained with an organism such as a sponge by taking the sponge and rinsing it quickly in distilled water and drying it either in air, when it would largely decompose, or heating it in an oven at 90° C., in which case it would take days to acquire a constant weight and give off a good deal besides water and incidentally retain a good deal of its water. Alternatively, it might be dried for weeks in a vacuum desiccator.

The method I now use is a modification of that of Dean & Stark for finding the percentage of water in oils. The apparatus is quite simple and is of a standard type which can be obtained from any dealer in chemical apparatus. For the smaller animals the apparatus is that figured in the diagram (Fig 1*c*). Assume for simplicity that one wants to find the percentage of water in some such animal as a mouse or a cockroach, both of which can be weighed on the balance without any appreciable error. The mouse is weighed, for preference in the distilling flask, and then covered with several times its volume of xylol. The whole is then heated over an electric hot-plate, and both xylol and steam are driven off and condensed and fall together into the graduated receiver. The two liquids are not miscible and the xylol floats on top of the water. Xylol distils at a temperature of just over 130° C., and so a good deal is driven off besides water, but any fats or oils remain in the xylol and do not affect the result. The point at which all the water has been driven off is clearly marked, for so long as water is coming off the xylol floating above the water is cloudy; distillation is therefore continued till a clear layer of xylol forms above the water. In actual practice it seldom takes more than an hour's distillation to drive off all the water.

Paraffin cannot be substituted for xylol as it does not have a constant boiling-point. This has been tested carefully, and at the end of an hour the boiling-point has usually risen to 160° C. and many of the tissues have begun to char. Glucose and starch are not affected by being distilled under xylol for a couple of hours, but cane sugar is and begins to char. In most cases it is sufficiently accurate to read off the volume of the water and take this as so many grams, but for more accurate determinations it is better to read off the scale, then empty the receiver completely and clean it. Then run in a little xylol and finally run in distilled water from a standard burette up to the previously observed scale mark. Then run the same volume of water from the same burette into a dry and weighed flask or weighing bottle and weigh again.

For larger animals the apparatus shown in the diagram (Fig. 1*d*) is used. It consists of an ordinary Soxhlet extractor. This is first boiled out with xylol and the syphon tube is then sealed by putting a small amount of mercury in the extractor. The distillation is carried out as before, but the water which is condensed collects at the bottom of the extractor. When the distillation is complete and the apparatus has cooled down, both mercury and water and some of the xylol are run out by means of the tap at the bottom of the extractor into a suitable graduated cylinder or possibly a standard flask. For ordinary work the heights of the top of the mercury and the top of the water are read, but for accurate work only the height of the water under the xylol is read, and then the mercury, water and xylol are run out. The mercury is dried with acetone and warm air and returned to the clean dry graduated cylinder. Some clean xylol is added and then distilled water is run in from a standard burette as before.

The process has many advantages apart from the great saving of time. Volatile substances such as oils which would come off below the temperature of boiling xylol are absorbed by the xylol. If a gas such as HCl comes off as the result of dissociation of the calcium and magnesium chlorides, it does not cause a loss in weight, for it dissolves in the water and the water is determined in the first instance by volume and not by loss in weight.

I recently determined the percentage of water in the ordinary hen's egg by this method. The distillation took less than an hour and the result obtained was 65·4 %, which agrees closely with the figure given by Lebbin, 65·16 % in 1900 as quoted in *Chemical Embryology* by Needham (Cambridge, 1931). Doubtless the percentage of water in a hen's egg will vary with age, etc.

With a sponge the technique is slightly different, but it is quite simple. First of all a rather extensive set of experiments had to be carried out to determine what weight of distilled water is to be obtained when 100 g. of the Plymouth Laboratory sea water is distilled under xylol by the method described above. The sea water in the tanks at Plymouth varies, but any serious alteration in its alkalinity is accompanied by a change in density, and this is automatically checked in finding the density or sinking factor of living aquatic organisms. Actually it was found that 100 g. of the laboratory sea water, having a density of 1·02518 at a temperature of 19·0° C., gave 96·8 (actually 96·796) g. of distilled water. This figure may or may not agree with the theoretical yield, but it includes the percentage experimental error which is far more important. The sponge is then weighed by the displacement method and returned to circulation for an hour. It is then transferred to the distillation flask, which has been weighed. If the sponge was known to weigh 50 g. and the contents of the flask weighed 100 g., there would be 50 g. of extraneous sea water present. If on distillation 80 g. of distilled water was obtained, that from the sponge would be $80-48·4$ g. Thus the percentage of water in a living sponge is found. In the modified Dean & Stark method which requires the Soxhlet extractor some flasks had to be made with specially wide mouths and ground-glass adaptors, but otherwise the apparatus is again of a standard type.

In passing it may be well to note that the special method of distilling sea water is of some interest apart from anything else, for it is not possible by any known means to take sea water and evaporate it to dryness and obtain a constant weight of residue. Sea water always contains both calcium and magnesium chlorides, and as the water is driven off these will dissociate and continue to give off HCl till both the calcium and magnesium are present as CaO and MgO. On distilling sea water below xylol a certain amount of dissociation always takes place and HCl is given off, but this makes no difference in the determination of the percentage of water, for as explained above the water is first measured by volume and not by weight. Contamination with xylol would in any case rule out the actual weighing of the water driven off.

THE PERCENTAGE OF SILICA AND NITROGEN

On distilling the sponge *Ficulina* under xylol it does not disintegrate but retains its shape in a rather remarkable manner. The sponge can then be removed, the xylol burnt off and the whole heated to redness in a silica basin. The residue is then digested with strong nitric acid and the silica finally filtered off on a Gooch crucible which is heated to redness for several hours and then weighed. Thus the percentage of silica is obtained. Similarly a weighed amount of living sponge can be digested with sulphuric acid and potassium sulphate and the nitrogen estimated by Kjeldahl's method. Calcite can be estimated by several methods, but so far the percentage of carbon has not been determined successfully.

RESULTS OBTAINED WITH THE DIFFERENT ORGANISMS

SPONGES AND THE DENSITY OF PROTOPLASM

	Density	Temp. ° C.	Calcite %	Density of protoplasm	Water %
Sycon coronatum	1·0925	14·6	7·88	1·0370	—
			Silica		
Halichondria panicea	1·068	15·0	5·08	1·0384	—
Ficulina ficus	1·0809	15·6	3·85	1·057	—
	1·077	15·6	3·98	1·056	—
	1·0805	16·5	5·506	1·049	81·6
	1·0815	17·0	5·35	1·0509	80·94

The above figures were those obtained for a number of sponges, but unfortunately it was not possible at the time to substitute gravimetric estimations for titrations. It is obvious that there is a great deal of work to be done in this direction, but it is felt that it must be postponed till more material can be obtained and longer periods devoted to it.

G. P. Bidder realized many years ago that if the density of a living sponge could be obtained and the percentage of either calcite or silica present as spicules estimated, it should be possible to obtain a close approximation to the density of the protoplasm itself. This assumes that a sponge is composed of little else besides spicules and protoplasm. We know that a sponge consists of other things, but since it is unlikely that their density differs greatly from that of protoplasm the above assumption is reasonably valid. On this assumption the density of protoplasm has been computed, the method being the following:

Sycon coronatum (Ellis & Solander)

Temperature throughout: 14·6° C.

Distilled water (filling density bottle)	50·9652 g.
Sea water (filling density bottle)	52·3331 g.
Sea water and sponge (filling density bottle)	53·0210 g.

Volume determination

Titrations 14·31 and 11·40 ml. with $N/10$ silver nitrate

$$\text{Vol. of sponge} = 50{\cdot}87 - \left(\frac{11{\cdot}40}{14{\cdot}31} \times 50{\cdot}87\right) = 10{\cdot}345 \text{ ml.}$$

$$\text{Density of sea water} = \frac{52{\cdot}3331 \times 0{\cdot}999190}{50{\cdot}9652} - \frac{0{\cdot}0012\,(52{\cdot}3331 - 50{\cdot}9652)}{50{\cdot}9652} = 1{\cdot}026055.$$

Weight of water displaced by sponge $= 10{\cdot}345 \times 1{\cdot}0260 = 10{\cdot}6145$ g.

Weight of water remaining in density bottle $= 52{\cdot}3331 - 10{\cdot}6145 = 41{\cdot}7186$ g.

Weight of living sponge $= 53{\cdot}0210 - 41{\cdot}7186 = 11{\cdot}3024$ g.

$$\text{Density of sponge} = \frac{11{\cdot}3024}{10{\cdot}345} = 1{\cdot}0925$$

Weight of calcite	0·891 g.
Percentage of calcite	7·88
Weight of silica	0·0351 g.

$$\text{Density of protoplasm} = \frac{W - (M' + M'')}{V - \left(\dfrac{M'}{2{\cdot}72} + \dfrac{M''}{2{\cdot}65}\right)}.$$

W, weight of living sponge; M'', weight of silica;
M', weight of calcite; V, volume of living sponge.

Density of calcite $= 2{\cdot}72$.

Density of quartz $= 2{\cdot}65$.

$$\text{Density of protoplasm} = \frac{11{\cdot}3024 - (0{\cdot}891 + 0{\cdot}035)}{10{\cdot}345 - \left(\dfrac{0{\cdot}891}{2{\cdot}72} + \dfrac{0{\cdot}035}{2{\cdot}65}\right)} = 1{\cdot}0371 = 1{\cdot}0371 \text{ at } 14{\cdot}6° \text{ C.}$$

I am greatly indebted to Dr Bidder for much advice and helpful criticism in connexion with these sponges.

CRUSTACEA

	Temp. ° C.	Sinking factor
Branchiopoda		
Chirocephalus diaphanus Prévost	11·3	1011
Artemia salina (Linn.)	10·0	988
Daphnia pulex (de Geer)	7·0	1017
Ostracoda		
Candona candida O. F. Müller	7·6	1025
Herpetocypris reptans Baird	8·8	1170
Copepoda		
Calanus finmarchicus (Gunnerus)	15·4	1029
” ” ”	15·4	1033
Anomalocera patersoni Templeton	16·2	1014
Diaptomus gracilis Sars	8·0	1023
Tigriopus fulvus (Fischer)	15·6	1060
Leptostraca		
Nebalia bipes (Fabr.)	7·4	1045
Amphipoda		
Gammarus pulex de Geer	20·0	1066
” ” ”	20·0	1088

	Temp. ° C.	Sinking factor
Mysidacea		
Hemimysis lamornae (Couch)	6·4	1075

Decapoda	Water %	Error by direct weighing %	Temp. ° C.	Sinking factor
Leander serratus (Pennant)				
Female (not in berry)	65·08	73·7	15·2	1098·4
,, ,, ,,	—	—	13·8	1086
Female (not in berry) (highly coloured)	73·14	—	15·2	1066
Female (in berry)	—	—	15·2	1068·8
First larva	—	—	15·2	1074
Palaemonetes varians (Leach)	—	—	10·8	1071
,, ,, ,,	—	—	10·8	1049
Crangon vulgaris Linn.				
Female (in berry)	—	—	17·2	1083
First larva	—	—	15·5	1052
,,	—	—	15·5	1039

Homarus vulgaris Milne-Edwards.

	Length in in.	Water %	Error by direct weighing %	Temp. ° C.	Sinking factor
Female late intermoult	7·5	66·8	—	16·6	1128
24 hr. after moulting	7·5	82·24	4·5	16·6	1050
Female, large encrusted	14·55	—	1·4	15·2	1137·1
Female, similar	14·55	—	2·6	15·2	1139·8
Female in berry	10·2	—	3·7	15·2	1129·3
Female in berry	10·5	—	4·2	15·2	1120
Male, shell clean	11·8	—	3·6	15·2	1140
Male, shell clean	8·4	—	2·4	15·2	1099

Embryos:	Water %	Temp. ° C.	Sinking factor
Fairly young: 2100 × 1862 μ 1980 × 1560 μ	51·2	15·2	1066·1
Slightly younger	43·24	15·2	1073
Much later, about to hatch: 2340 × 1980 μ 2250 × 1920 μ	—	15·2	1047

Portunus depurator (Linn.) Palmer

	Temp. ° C.	Sinking factor
Male, 4 specimens	16·6	1143
Female, not in berry	16·2	1133
Same specimen, in berry	16·2	1123
Embryos:		
White: 16 blastomere stage	15·2	1098
8–64 blastomere stage: Size 320 × 330 μ 340 × 340 μ	15·2	1090·2
Red	15·2	1070
Black, just before hatching	15·2	1047
1st larva (pre-zoaea)	15·2	1053
Megalopa (29 specimens)[1] 1.092	15·2	1065
Portunus puber (Linn.) Palmer: Males, 2 large	16·2	1136·7
Carcinus maenas (Pennant): Male	16·6	1177

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[1] Weight of a single specimen 0·0024 g.

Cancer pagurus Linn.

	Width of carapace in cm.	Error by direct weighing %	Temp. ° C.	Sinking factor
Female	11·21	2·5	5·6	1091
Female	20·15	1·16	15·2	1176
Female, 2 hr. after moulting	10·68	—	16·0	1023·6
Female, same crab as above but 1 week later, abdomen still soft	12·7	—	15·2	1054
Male	6·8	5·6	15·2	1218
Male	10·13	3·0	5·6	1112
Male	10·5	2·5	5·6	1187
Male	15·5	0·98	5·6	1230
Male	23·75	1·2	15·2	1226

Fish

	Temp. ° C.	Sinking factor
Conger vulgaris Cuvier (27 cm. long)	10·0	1000
Gasterosteus aculeatus Linn.	10·0	1003
” ” ”	20·0	1002
Crenilabrus melops (Linn.)	13·8	1004·5
Lepadogaster bimaculatus (Donovan)	15·0	1047
Pleuronectes platessa Linn., 5 small specimens	6·8	1036·3
Cottus bubalis Euphrasén: Female	6·4	1046
Male (water 72·56 %)	6·4	1054

Scyllium catulus Cuvier (nursehound)

Two young male specimens were obtained from the trawl. One (A) was weighed and then distilled under xylol and the percentage of water estimated, while the other (B) was kept alive in the aquarium and weighed at intervals. Another specimen (C) was a young female. There is apparently no means of telling the age of the Selachii. One large male was brought in to the laboratory having a weight (by direct weighing) of 5763 g. and a length of 112 cm., while another weighed 4798 g. and had a length of 105·4 cm. By observing the growth rate of the small specimen (B) it was hoped to estimate the age of these larger fish, but unfortunately the death of the specimen after being kept alive for nearly 18 months put an end to this work for the time being[1]. The following results were obtained:

	Age weeks	Length cm.	Weight g.	Volume ml.	Density	Water %	Temp. ° C.	Sinking factor
A	—	24·1	41·34	38·51	1·0734	69·71	16·0	1046
B	—	21·6	31·36	29·32	1·0695	—	16·0	1042
B	17	24·4	49·07	45·53	1·0773	—	5·6	1047
B	45	27·5	72·74	67·68	1·0747	—	15·2	1048
B	52	28·0	92·13	85·67	1·0754	—	15·2	1048
C	—	28·5	68·85	63·89	1·077	—	16·2	1049·7
Scyllium canicula (Linn.) (dogfish)								
Female		55·0	548·92	510·1	1·0761	—	17·2	1049

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[1] The figures under B show that after one year there had been the following changes:

Increase in length	29·5 %	Increase in weight	193·8 %
Increase in volume	192·2 %	Increase in density	0·55
Increase in sinking factor	0·62 %		

Miscellaneous

	Water %	Temp. ° C.	Sinking factor
Amphioxus lanceolatus (Pallas)	—	13·2	1040
Nereis diversicolor O. F. Müller	—	7·4	1045
Nereis virens (Sars)	—	9·6	1033
Anemonia sulcata (Pennant)	88·19	9·5	1017·6
Frog, *Rana temporaria* Linn:			
Tadpoles, 14 days old	—	9·6	1014
			Density
Ovary, just before ova enter the oviduct	—	7·4	1·0962
" " " "	—	7·8	1·0987
Testis, at breeding season	—	8·0	1·0596
" " "	—	9·2	1·0596
" " "	—	9·8	1·056
Hen, *Gallus*, yolk from egg	—	15·0	1·0252

Discussion of Results

Direct Weighing

The displacement method of weighing an aquatic organism is a lengthy one, but there seems to be no alternative way of obtaining accurate results. I very much hoped that experiment would show that with the larger animals the error due to direct weighing, or weighing the animal wet on the balance, would be small and more or less constant; but this is not so. It would be possible to remove a great deal of the adhering water by the application of a cloth or filter paper, but this does not solve all the difficulties. By draining the animal for a definite time it is possible to keep the error on the one side, and certainly if the error were approximately constant much time could be saved; but the figures show that this is not possible. Thus, two large lobsters of the same length and neither of which was in berry gave widely different percentage errors with direct weighing. With smaller crustacea in berry the percentage error may be enormous. Reference to the figures will show that in a prawn in berry the error was nearly 74 %.

Swimming

An animal can only swim freely so long as it can support itself, and in all the organisms examined so far, with the exceptions of *Artemia* and the conger, a definite effort must be made by the animal to keep itself up. I have been able to make one determination only of the sinking factor of *Artemia*; it appears that when it is swimming in strong brine, which is its normal habitat, it has to exert a muscular effort to keep itself *below* the surface. This state of affairs is not confined to *Artemia*, for in addition to many of the aquatic insects which take a supply of air below the surface of the water with them, the common stickleback, *Gasterosteus aculeatus* may be mentioned. This fish in fresh water has a sinking factor of 1002, which means that it must make an effort to keep itself off the bottom. Frequently the fish can be seen resting on the bottom, and it often feeds on crustacea, many of which have a high sinking factor. I have observed this fish after a big feed of ostracods unable to lift itself up at all. The stickleback can also be transferred suddenly from fresh water to sea water without any apparent injury, and this means that for a period the fish is less dense than its environment or in other words its sinking factor is less than 1000. I have observed that when treated in this manner the fish exert a great deal of energy in keeping themselves down, and it takes about 6 hr. before they are again capable of resting on the bottom.

The amount of work which must be done by a fish to keep itself off the bottom must depend on its sinking factor and, as stated above, if this is 1000 no effort is required. It is possible to show on theoretical grounds that it is not possible for an aquatic organism to remain in a state of hydrostatic equilibrium with its environment; but it appears that in the conger the difference between its density and that of its environment is less than 1 part per 1000.

The limitation of cilia as organs of locomotion was pointed out by Gray in 1928 (*Ciliary Movement*, Camb. Univ. Press), and it should be clear that cilia can only sustain and propel an organism provided its sinking factor is below a certain figure. What that figure is remains to be determined. The first larvae of echinoderms certainly have a low sinking factor and this is true of trochophores, but I look forward with great interest to finding the highest sinking factor attained by free-swimming veligers or the later larvae of echinoderms or any organism supported by cilia alone. What has been said about cilia is also true of limbs or other appendages moved by muscles. So far as fish are concerned it appears that the limit has been attained by the dogfish or nursehound, and the figure appears to be very close to 1050; but it must be remembered that the method of obtaining the correct sinking factor has only just been perfected. Few figures are available for dogfish, and at present living dogfish cannot be obtained at Plymouth. No determinations for the skates or rays have yet been made, but it is interesting to note that the sinking factor of such teleosts as the young plaice, *Lepadogaster* and *Cottus*, which only swim with difficulty, is below that of the free-swimming dogfish.

With crustacea the sinking factor attains a far higher figure. 1098 has been recorded for the prawn, *Leander*, which normally swims by means of its pleopods, while 1140 has been obtained for the lobster, an animal which is certainly capable of raising itself off the bottom even if it does not actually swim. *Portunus* reaches an even higher figure, 1143, but neither of the two animals last mentioned can be described as pelagic, and it is doubtful if this term can be applied to prawns or shrimps which spend a great deal of their time resting on the bottom. Truly pelagic malacostraca are difficult to secure, especially at the present time, but they will probably be found in due course among the euphausids or mysids. It is interesting to note that *Hemimysis lamornae* has a sinking factor of 1075, while that of the first larva of the prawn, which is certainly pelagic, is 1074.

A point of considerable interest arises with regard to crustacea in berry, and the work may throw considerable light on the habit of carrying the eggs or embryos which is so prevalent among the crustacea. At first sight it would appear that the female is considerably hampered by having to carry this extra load, but this does not really represent the actual fact. In all the cases so far investigated the embryos have a sinking factor which is less than that of the adult and, thus, while a prawn in berry does actually weigh more than one not in berry, yet her sinking factor in berry is less than when she is out of berry. The load of eggs or embryos may impede her rate of swimming, but it has the compensating effect of causing her to make less effort to keep herself afloat. Thus it may be an advantage rather than otherwise for the female to carry her young. This, however, cannot be the cause of her doing so, for the far heavier crabs, which are quite incapable of swimming, also carry their eggs.

Reference to the figures on p. 568 will show that the sinking factor of the crustacean eggs and embryos is high. Cilia do not occur in the crustacea, but if they were to be found we should expect them first to occur in the early embryonic stages. The segmentation in the early stages of *Portunus depurator* is equal and holoblastic and the blastulae appear to be as symmetrical in their formation as those of *Echinus esculentus*. They are, however, much larger and never become free swimming by means of cilia; their sinking factor is in fact so high that they could not in any event be supported by cilia. It is true that the early embryos of *Portunus depurator* are considerably larger than those of *Echinus esculentus*, but the embryos of such organisms as *Cyclops* and *Diaptomus* are much smaller than many of the ciliophora or the flagellated organisms like *Volvox globator*. It appears then that there is something in the crustacean embryos which makes them abnormally heavy, but what this substance is apparently remains to be investigated.

Statements are made in many standard text-books that the density of the ovum in general is determined by the amount of yolk it contains, a large amount of yolk causing a high density. This, however, is incorrect, for the density of the yolk of the ordinary hen's egg has been obtained and found to be 1·0252 at 15° C., which agrees fairly well with the figure given by Baudrimont & de St Ange (1846), as quoted by Needham in *Chemical Embryology*, namely, 1·0288–1·0299. This means that if the yolk of the crustacean egg is in any way comparable to that of the hen a large amount of yolk should decrease the density or lessen the sinking factor, since the density of the yolk of the hen's egg is less than that of Plymouth sea water and less than that of protoplasm itself.

The density of the cytoplasm of the eggs of *Arbacia* according to Heilbrunn as quoted by Gray (*Experimental Cytology*, Cambridge, 1931, p. 63) is 1·03583. This is lower than the lowest figure which I obtained for the density of protoplasm in sponges, but even so it is higher than the density of yolk. Reference to Heilbrunn's paper reveals to my mind a rather serious objection, for to quote Gray: 'The specific gravity of the granules was determined by shaking the eggs to pieces; the resultant suspension was then centrifuged into sugar solutions of varying specific gravity to find a solution in which the granules just pass to the bottom of the solution.' But solutions of sugar which vary in density must also vary in osmotic pressure. A hypertonic solution of sugar would withdraw the lightest substance in the granules, namely, water. This point seems to have been overlooked.

The eggs[1] of *Portunus depurator* and *Homarus vulgaris* appear to be very dense to begin with, and the sinking factor gradually becomes less and less, probably due to the absorption of water, till they assume a sinking factor of about 1050. At this stage the larvae become free, with a sinking factor of practically the same figure, and muscular limb movement readily supports them.

In passing it is interesting to note that the sinking factor of *Calanus* is so low that it is probably lower than that of its embryos. It is also an organism which undergoes extensive vertical migrations, and it therefore appears to be significant that the eggs are not carried by the female, at any rate in the later stages.

Nearly all the work on crustacea is, however, hampered by their undergoing ecdysis. A crab just after moulting had a sinking factor of 1023, while a week later its sinking factor was 1054. Months later it would attain a figure of 1230 according to the data already recorded, and until there is some means of ascertaining the state of the intermoult the use of the figures for the sinking factor is somewhat restricted. It seems that a rather laborious piece of work is indicated, namely, that of isolating a number of crustacea and weighing each individual periodically. An attempt was made to ascertain how far the sinking factor of *Palaemonetes varians* was effected by its migrating from sea water into brackish water; but the experiment consisted merely of finding the sinking factor of some individuals kept in brackish water and comparing it with data from those kept in sea water. The figures showed nothing and clearly any change would be masked by variation due to the state of the intermoult. An adult female freshwater crayfish, *Potamobius pallipes*, was investigated and compared with a young female lobster, neither being in berry. The result is given below, but it is doubtful whether it means very much at present.

	Crayfish, adult female	Lobster, length 7·5 in.
Sinking factor	1130	1128
Water	71·05 %	66·68 %

The highest figure reached by any freely moving animal is 1230 for the crab, *Cancer pagurus*, with a width of carapace of 15·5 cm. It is interesting to note that a much larger specimen with a width of carapace of 23·75 cm. gave 1226, while a much smaller specimen with a width of carapace of only 6·8 cm. gave 1218. The crabs appear to have higher sinking factors than the lobsters, but in spite of this they seem to have attained far greater activity on land.

No attempt will be made to discuss the function of the so-called swim-bladder of the teleost fishes; it is a fact that those fishes with a swim-bladder have a low sinking factor while those without it have a high one. Reference to the figures will show that the sinking factor of adult dogfishes and nursehounds appears to be remarkably constant, namely, 1050 ± 1.

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[1] The word *egg*, though it is the one in general use, should really be replaced by *embryo*.

The Effect of Spines in Crustacea

A very noticeable characteristic of many of the pelagic crustacea, both fresh water and marine, is the outgrowth of the integument in the form of spines or setae, and the function of these has been the subject of a good deal of discussion. By some they are supposed to have a buoyancy effect, while it has also been suggested that their effect is to maintain or restore the centre of gravity of the organism to a certain position. Clearly the term *buoyancy* cannot be used of an organ or tissue which has a greater density than that of the environment, and the term should be restricted to such structures as bladders containing gas or possibly to oil-drops. I have discussed the effect of these spines on the centre of gravity in a previous communication and given my reasons for considering this theory untenable (Lowndes, A. G., *Proc. Linn. Soc. London*, Ses. 150, 1937–38, 25 March 1938, pp. 62–73). The chief effect of these spines is to slow down the rate of sinking. Spines and setae can have another effect however, for it is known that pelagic crustacea such as *Calanus* and *Diaptomus* as well as the nauplius and many decapod larvae have a slow gliding method of propulsion to which is added a sudden leaping or escape movement. *Calanus* itself when quiescent remains with its antennules extended and its body practically vertical. The sudden leap due to the action of the thoracic limbs which are otherwise motionless causes the animal to dart away suddenly in a plane at right angles to the vertical. If it darted off in the vertical direction when it was near the surface it would clearly leap out of the water (this actually happens in *Anemalocera patersoni*). *Calanus* is able to hang vertically by means of the long and characteristic plumose setae on the antennules. The effect of these setae thus is to cause the animal to sink slowly and in the most advantageous position so far as its escape movement is concerned.

SUMMARY

The method by which the weight, volume, density and sinking factor of living aquatic organisms can be determined has been extended. By sinking factor is meant the ratio of the density of the organism to that of its environment at the time of the actual determination. The density of the environment is always raised to 1000, and the density of the organisms computed on this basis.

A quick and accurate method for determining the percentage of water in a living organism has been described and discussed.

On the assumption that a sponge consists of protoplasm and spicules only, the density of the protoplasm has been computed.

The sinking factor of several fish and of the embryos of some crustacea at different stages has been determined. One effect of the swim-bladder in teleost fishes is to reduce the sinking factor and thus facilitate swimming.

The effect of the sinking factor on swimming in general and the effects of spinous outgrowths in pelagic crustacea are discussed.

THE RELATION OF *GAMMARUS ZADDACHI* SEXTON TO SOME OTHER SPECIES OF *GAMMARUS* OCCURRING IN FRESH, ESTUARINE AND MARINE WATERS

By E. W. Sexton, F.L.S.

From the Plymouth Laboratory

(Plates I–III, Text-fig. 1)

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INTRODUCTION

Gammarus zaddachi, the species under discussion in this paper, is one of the most widely spread of the amphipods which inhabit the estuarine waters of northern Europe. It has been recorded in the river mouths and adjacent seas from the White Sea, along the Baltic, the coasts of Scandinavia, Prussia, the Netherlands, Great Britain and France and from the Crimean region also. Most of the early records are under the name of *G. locusta*, but many of these I have been able to revise, having had access to several large collections, housed in the British Museum, the Königsberg and Hamburg Museums and others.

This species has always been confused with *G. locusta*—why, it is difficult to say, as the characters are quite distinct. Their habitats also are different, *locusta* being a marine species, and *zaddachi* typically brackish water, though there may be a little overlapping at the seaward limit of its range. The discovery referred to below, of the difference in the development of the sensory armature of the cuticle in relation to the environment, may perhaps account for some of the confusion which has grown up round the two species, though not for all. Some may be due to incorrect identifications and the consequent incorrect data on distribution. In view of the immense range of *G. zaddachi* and its recognition and acceptance as a typical salinity indicator in ecological work, it is important that the whole subject should be cleared up once for all and the present paper is an endeavour to this end.

G. zaddachi was first established as a distinct species in 1912, when I found and described ten specimens of it in a collection of Crustacea taken by Herr W. Klie in the harbour of Bremerhaven. I named it after Zaddach (1844), who was the first to draw attention to its difference from *G. locusta*, although he recorded it, with a query, under that species. I was enabled, however, before publishing my description, to examine Zaddach's original specimens preserved in Königsberg Museum, and thus confirm the distinctions between the two species.

The material from several collections available to me at that time consisted of about 2000 specimens of *G. zaddachi* taken in many of the northern European estuaries and ranging from the fresh water at the upper end, through variations of salinity to the mouth of the river where it entered the sea.

During the course of the investigations on the species a remarkable fact came to light, viz. that the difference in habitat was correlated with a difference in the external appearance, though the structure in all the forms was identical. The animals taken at the seaward limit of their range, the typical 'saline' estuarine form, were delicate and pellucid, the chitin so thin as to be semi-transparent, with comparatively very few hairs[1]; in consequence they presented a more spinose appearance when compared with the hairy 'freshwater' form. The latter, living in fresh water in the upper reaches of rivers and in lakes, were more solidly built, opaque, with thicker chitin in the cuticle, and with dense clusters of long fine hairs on the appendages in addition to the spines. (For description and figures see pp. 593–602, Pls. I–II.)

In fact, on taking typical samples of the two forms from the extremes of the species range, the difference in their appearance was at first glance so great as to suggest two distinct species. Further observation, however, proved it to be entirely due to the greater development of the epidermal outgrowths (the 'sensory armature') in freshwater conditions. It must be emphasized that structurally the two forms are identical, and that it is not possible to find any point of distinction other than the increase of hairs. A further proof of their being the same species is provided by the specimens which I have called 'intermediates'. These occurred in different localities in varying salinities some with more hairs developed, some with less.

It may be said as a general rule that as the salt content of the water increases so the development of the hairs decreases. A very interesting instance of this is shown in a series of samples from the Elbe, ranging from the typical 'freshwater' hairy form taken in the Hamburg Water supply, with numbers of intermediates in the early stages collected at different stations down the river, to the typical 'saline' form at the farthest seaward station in the estuary at its mouth. This distribution has since been amply confirmed in several English rivers, where good series of dredgings have been made throughout the whole length of the estuaries.

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[1] In the general descriptions throughout the paper, the word 'hairs' is used as a comprehensive term to include all the varieties of sensory equipment such as the finest delicate hairs, and setae of all grades to strong bristles, as distinct from the spinose armature.

The more exact methods employed by recent workers, such as Serventy, Crawford, Spooner, Reid, Goodhart, and others, give clear-cut results as to the limitations caused by tidal influence on the distribution of the common north European *Gammarus*, *G. locusta*, *pulex*, *duebeni*, and *zaddachi*. The importance of these species in modern ecological work lies in their use as salinity 'indicators', and naturally any confusion as to their identity would nullify the value of the results.

As has just been said there has been great confusion in the past, which unfortunately persists to the present day. One of the principal causes seems to be the perpetuation of the errors of the old records in the identification of species, through acceptance of their statements without verification; and this has naturally given rise also to errors in the records of the distribution of the species. Inaccuracies of this kind have been handed down in the synonymy of a species by one Crustacea worker after another.

It is not surprising that in the early days mistakes were made. The number of species known to science was very small, and the descriptions and figures, while definite enough to distinguish one of these from the few others, proved quite inadequate for the purpose when applied to the numbers of closely allied forms found later. These forms could easily be fitted into the generalizations of the original diagnosis of the genus, but the finer specific distinctions were not noted, specimens of different species being only too often all labelled *G. locusta* if found in the sea, or *G. pulex* if taken in fresh water.

G. locusta, the oldest established species of *Gammarus*, was given by Linnaeus as the type of his genus; later authors, following him, redescribed the species, e.g. J. C. Fabricius (1775), O. Fabricius (1780), G. Montagu (1808), H. Milne Edwards (1830), Rathke (1836), Zaddach (1844), Brandt (1851) and many others, but as they were not all dealing with the true *locusta*, their accounts have added to the confusion instead of clearing it. In fact, it is impossible to-day to know with certainty which species is referred to by these early writers without having access to the original collections and examining the actual specimens described by them.

Another cause of confusion may perhaps be found in the theories formulated by certain carcinologists in their efforts to solve the species problem—theories built on very insecure foundations. An example may be given from Schellenberg (1937). The theory he puts forward seems to be that a new variety of a species may be formed directly by new modifications of its environment, and that conditions which work in the same way will produce similar changes, so that forms resembling each other are to be found living discontinuously in places far apart.[1] In the case he is arguing he considers that by a lowering of the salinity *G. zaddachi* is produced as a brackish-water variety of the marine *G. locusta*. He ignores the facts that the specific characters distinguishing *zaddachi* are as clear-cut and definite as those of *locusta*, and that the resemblances are simply generic. Even where the areas of their distribution overlap a little in the marine zone, *zaddachi* is as distinct from *locusta* there as it is from *G. pulex* in the fresh water at the other end of its range (see also p. 592).

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[1] Schellenberg (1937, p. 514) summarizes it thus: 'Mehrere Befunde bestätigen den Einfluss der äusseren Bedingungen auf den *Gammarus*-Bau. Gleichartig wirkende Bedingungen haben konvergente Formen hervorgebracht, so dass übereinstimmende Formen, diskontinuierlich an weit entfernten Stellen auftreten.'

Perhaps a further source of error may lie in the fact, pointed out to me by Prof. J. M. Pirlot (in litt., 6 February 1939), that even now another species, quite distinct but hitherto undescribed, frequenting a lower salinity than *locusta*, is still being mistaken for it and recorded under its name. He had described and figured it ready for publication just before the outbreak of war. Knowing how salinity conditions affect and limit the range of a species, I think it will be found later that many of the records which now puzzle ecologists, of the occurrence of *locusta* in unusually low salinities, can often be explained by the presence of this second species.

In the present paper I have limited my acceptance of the records of *G. zaddachi* to those that I could verify myself, though no doubt many others are correct in identification. There still seems to be such difficulty in recognizing the two forms of the species that I have described and figured them both in detail. The illustrations are taken from the original specimens, the 'saline' from Zaddach's collection at Königsberg, the 'freshwater' from the Hamburg water-supply pipes. They have been compared with hundreds of specimens from British rivers and estuaries and have agreed to the smallest detail. I have also noted the most striking points of difference from other species, *locusta*, *pulex*, *duebeni* and *wilkitzkii* (pp. 600–1).

I am greatly indebted to my daughter and to Dr E. J. Allen for the help I have received from them in the preparation of this paper.

Historical Review

The principal papers dealing with *G. zaddachi* are described below in chronological order. In tracing back the history of the species its records were found to be so involved with those of *G. locusta* from the earliest beginnings that it was impossible to extricate them without examining the specimens described. This I have been unable to do farther back than 1836 (Rathke).

The first reference to *G. locusta* is in an account by Linnaeus (1745, p. 260) of an amphipod taken on the seashore at Gothland, and named by him *Cancer macrourus coeruleus*. In the 10th edition of his *Systema Naturae* (1758), as also in later editions of this work, he has recorded it as *C. locusta*. For many years Linnaeus's work was the standard authority on the Crustacea, and the tendency of workers following him seems to have been to identify any marine *Gammarus* with his *locusta*, any freshwater one with his *pulex*. In an endeavour to define the specific characters with more exactitude, some of them amplified his description, e.g. J. C. Fabricius (1775, p. 418, under the name of *Gammarus* *locusta*, syn. *Cancer locusta* Linné); O. Fabricius (1780, No. 231, *Oniscus pulex* syn. *Cancer locusta* of Linné); Montagu (1808, p. 92, *Cancer* (*Gammarus*) *locusta*); H. Milne Edwards (1830, pp. 367–9 as *Gammarus ornatus*, and 1840, p. 44).

From certain discrepancies in their accounts it can be seen, however, that they were dealing not with one and the same species but with several different ones. Four names, Linnaeus, Fabricius, Montagu or Milne Edwards, are quoted indiscriminately by later writers as the authority for the species *locusta*.

1836 **Rathke** described as *Gammarus locusta* an amphipod taken by him in the Black Sea, on the eastern half of the Crimea, living on the sand and under stones where the sand was still moist. He stated that, as he had no detailed characterization of the *G. locusta* which occurred in England and France, he might be mistaken in his identification, and he would therefore give a close description. The different appendages are described with notes on their proportions, size and colour, and figures given of the 2nd gnathopod, peraeopod 5, urosome, and telson. The figures are not distinctive, but fortunately Rathke's specimens were preserved at Königsberg Museum. Two tubes of them were sent to me for examination, the one, labelled '*G. locusta*, Krim, Rathke', contained three males of the true *G. locusta* (L.); the other marked '*Gammarus*, Krim, Rathke' had three *G. pulex* in it, and one ovigerous female *G. zaddachi*.

1843 **Rathke** (pp. 67–8) in this work records *G. locusta* Montagu from several places on the west coast of Norway, from Christiania, and Droeback at the head of the Fjord, and from near Danzig in the Baltic. He compared them, and found no difference between them, beyond the fact that the eyes in the Danzig specimens were rather broader in relation to the length of the animals. Neither could he see any further difference when comparing them with his specimens brought back from the Black Sea, except for a greater length of the hairs on the 2nd antennae of the Crimean animals, and a slight variation in the proportionate breadth of the rami of the 3rd uropod.

Fortunately, some of his specimens were still in Königsberg Museum, labelled '*Gammarus locusta*, Montagu, Norwegen; Rathke'. I examined the fifteen preserved. They were all fine examples of *G. zaddachi*, the 'saline' form, 9–22 mm. in length, males and ovigerous females.

1844 **Zaddach** (pp. 4–6) recorded a species of *Gammarus* from the Prussian coast, which he attributed, though with a query, to *G. locusta* Fabr. as described by Milne Edwards (1840, p. 44). He pointed out that his specimens differed from this account, notably in two characters, viz. the presence of clusters of long hairs on both the antennae, and the proportions of the rami of the 3rd uropods. He stated that if Milne Edwards's characterization were accurate, then this description of his Prussian animals would constitute a new species of the genus *Gammarus*. It is interesting to find Zaddach had noted the two most striking distinctions between the true *locusta* and these specimens from the Prussian coast. He was evidently reluctant to institute a new species, perhaps because, having investigated the collection of 'the celebrated Rathke', from the Norwegian coasts, he had compared the '*G. locusta*' represented there with his own animals and had found them identical in structure. That he was right in making this statement has just been shown above—the Norwegian specimens labelled *G. locusta* by Rathke were in fact all *G. zaddachi*.

I was able to examine Zaddach's collections (see Sexton, 1912) and establish beyond doubt the distinction of the species. I therefore named it in honour of Prof. Zaddach, as he was the first to draw attention to the characters peculiar to it.

1851 **Brandt** (pp. 132–5), in reporting on von Middendorff's Siberian collections, found a single specimen of *Gammarus* from the Doschkander River which flows into the Sea of Okhotsk. He placed it under the Section 1. A. *a.* of Milne Edwards (*Hist. nat. d. Crust.*, 1840) defined as having the inner ramus of the 3rd uropod as large, or at least more than half as long as, the outer: he named it *G. locusta* with a query. He would, he said, have unhesitatingly attributed it to that species (the *G. locusta* of O. Fabricius) but for some differences in the proportions and greater hairiness of the antennae and the shape of the uropods, and also because of lack of knowledge of the structure of the 3rd pair, which was missing.

Brandt made a great effort to clear up the confusion in the synonymy of *locusta*, as he had found that several obviously different species had been included under this name. He traced the history of the species from the original diagnosis of Linnaeus, and discussed the various authors who had afterwards dealt with it, Roesel, Klein, Sulzer, Herbst, Pallas, Frisch, Otto Fabricius, J. C. Fabricius, Montagu, Kröyer, Leach, Desmarest and Rathke. The three last, he said, had accepted Montagu's determination (and this is borne out by Rathke's specimens being labelled '*G. locusta* Montagu'). He then referred to Milne Edwards's description of the species and the discrepancies between his account and those of O. Fabricius, and of Montagu. In discussing Zaddach's (1844) *Gammarus* from the Baltic he agreed with him that it could only doubtfully be assigned to *locusta* Fabr., since it showed several variations from Milne Edwards's description.

His conclusions throw little light on the subject, for they are based on the very inadequate descriptions and figures of the earlier authors, and not apparently on any of the actual specimens; but at least his work shows that by this time the need for more exact specific distinctions or definitions was clearly recognized.

1862 **Spence Bate** (p. 206, pl. 36, fig. 6) in the British Museum Catalogue, included under the name of '*Gammarus Locusta*' all the previous records, Linnaeus, Montagu, Milne Edwards, Rathke and Zaddach amongst others. He described the rami of the 3rd uropod as 'subequal'.

1863 **Bate** and **Westwood.** On p. 378 *G. locusta* is figured, practically the same drawing as in the British Museum Catalogue. The description is more detailed, and an effort is apparently made to combine the conflicting statements of the previous observers, without any realization that they related to different species. For instance, it is said 'the antennae have the peduncles strongly hirsute' (i.e. like *zaddachi*) but the figure shows them almost glabrous (i.e. like the true *locusta*); and the 3rd uropods 'have the branches subfoliaceous and nearly of one length, the inner one sometimes one-fourth or one-third smaller than the outer one' (i.e. including characters of both *locusta* and *zaddachi*).

1873 **Möbius** (pp. 118–19) recorded the occurrence of *G. locusta* L. from thirty-four stations in the Baltic, with the depths and nature of the ground on which the amphipods were found. As references he gave Linnaeus's *Syst. Nat.* and Bate & Westwood's description mentioned above. Some of these records no doubt apply to the true *locusta*, but that others are certainly of *zaddachi* I have been able to prove by examining the collection preserved at Königsberg Museum. The tubes sent to me were all labelled *G. locusta* and included several from the localities given by Möbius. One of these, marked '*Gammarus locusta* L. Kiel, Möbius, 1872, No. 6526' contained three specimens, a male and two female *zaddachi*, 13–15 mm. in length; two tubes from Zoppot, one also dated 1872, with forty-five *zaddachi* (saline form); three from the station near Memel with seventeen *zaddachi* (saline form); and mixed dredgings from Danziger Bucht, Zoppot, Hela, etc., various depths 1–25 m., 168 specimens, of which nineteen were *locusta* and 149 *zaddachi* (saline form).

1878 **Zaddach** (pp. 27–32) in this paper gives a full description of the species which he had previously identified as *G. locusta* Fabr. with a query, but which he now considers to be unquestionably that species. In his list of references he cites Fabricius, Spence

Bate, and Möbius. He gives a detailed account, with numerous figures, to illustrate the structure, the reproductive organs and the differences between male and female, and between the adults and the young and the changes at the various stages of growth. Of the two distinctive specific characters which he had emphasized in 1844 he mentions the long setae on the antennae, though without drawing particular attention to them; but, in discussing the second character, viz. the proportionate length of the two rami of the 3rd uropod, he affirms that his specimens differ definitely in this from the species character given by Spence Bate and Milne Edwards. Instead of 'the 2 rami of an almost equal length' they have the inner ramus the shorter of the two, about three-quarters the length of the outer ramus in adults and even shorter in the young.

Zaddach's specimens were preserved in the Königsberg Museum collection. I have examined thirty-six of his tubes of *Gammarus*, thirty-two of which were labelled *G. locusta*. They contained 648 specimens from a large area extending from the open sea near Memel, along the Baltic coasts westward to the Gulf of Danzig and Putziger Wick, and from near the shore to a depth of 25 m. Many rivers drain into this area, e.g. the Niemen, Pregel, Vistula, and the salinity is therefore low. It is interesting to note in confirmation of the statement previously made with regard to the range of the two species being strictly limited by the salinity that the seventy-one *locusta* of Zaddach's collecting were found in the higher salinity of the open sea, and the 577 *zaddachi* saline form, in the estuarine waters.

1886 **Kraepelin** (pp. 13–25) gives an account of the Hamburg water-supply system, which at that date drew its water from the Elbe above Hamburg, and of the living things that were able to enter it direct from the river owing to the lack of a central filter-plant. He took many samples of the contents of the underground pipes with the idea of perhaps finding blind forms living in the darkness, or modifications of the river animals caused by their life underground. Though unsuccessful in this, he obtained no less than fifty different genera in the fauna. Amongst the Edriophthalma he mentions *Asellus aquaticus* in all the samples, as occurring in thousands, and second to it only in numbers '*Gammarus pulex*'.

He points out that though the animals enter from the river, there is a dissimilarity between the faunas of the river and the supply pipes; that all air breathers and vegetable feeders perish in the latter, and only those survive which are provided with gills and feed on detritus. For these the conditions of life in the pipes are much more favourable than conditions in the river, the pipe fauna enjoying a much greater abundance of food, protection from its enemies, and only slight variations of temperature.

Some of this material collected by Kraepelin, and by the Director of the waterworks, was sent to me for examination. The specimens, all labelled '*Gammarus pulex*', numbered seventy-nine, and were all the typical freshwater form of *G. zaddachi*, the largest measuring 20–22 mm. in length. The illustrations of the freshwater form (Pls. I, II) were taken from one of these specimens.

1889 **Hoek**,[1] in preparing his list of the crustacean fauna of the Netherlands, was faced with the difficulty of the conflicting records of the characters and distribution of *G. locusta*. The earlier writers had accounted for the confusion by stating that the species was an extraordinarily variable one. Hoek was able, however, to show that this was caused, not by any variability of character or habitat, but by the fact that several distinct species had been included under the one name, *locusta*. He made a careful study of numbers of specimens from different localities, and as a result divided them into four well-defined forms, as follows:

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[1] My thanks are due to Mr Van de Kasteele, the Dutch Consul in Plymouth, for his kindness in translating Hoek's paper.

(1) The 'true *G. locusta*', living in the sea near the coast, farther out than *marinus*, the typical coast form;

(2) 'Variety A', inhabiting brackish water, found by Hoek himself in the Haringvliet and other places at the mouth of the Rhine, and the Waal;

(3) 'Variety B' (= *G. pulex* Fabr.), from perfectly fresh water in brooks and ditches; and finally

(4) 'Variety C', another brackish form, distinct from variety A, collected by Ritzema Bos in 1871 in a slightly brackish ditch near Warffum. This was later identified by Sars as '*G. duebeni* Lilljeborg'.

All four forms are well characterized with figures of the head, pleon armature, 3rd uropods and telson of each. I have compared Hoek's description of the characters of variety 'A', and its distinctions from *locusta* with *G. zaddachi* and found the two forms in such complete agreement (see p. 597) that there seems little doubt but that they are identical. The habitat also, with the salinity, is where one would expect the saline form of *G. zaddachi* to flourish.

Hoek found 'variety A' very common in the Haringvliet, at Hollandsch Diep above and below the bridge at Moerdyk in 4–7 fm., and at Nieuwe Merwede, where, he says, the salt content was somewhat different in these three differently named parts of the same river. The highest salinity he recorded was in the Haringvliet, 8·9 °/₀₀ at 6° C., though usually it is here much lower, from 3 to 3·2 °/₀₀. In the other places named it varied from about 1–0 °/₀₀. He was unable to extend his investigation farther up the river, though he considered it probable that such animals might exist there, and that it would be very important to ascertain to which variety they belonged.

1890 to 1894 **Sars** (p. 499, pl. 1 and pl. 176, fig. 1) describes the true *G. locusta* of Linnaeus, figuring it as the type of an amphipod. He was the first to give an accurate representation of the whole of its structure. He identifies Hoek's 'variety B' with *G. pulex*, and 'variety C' with *G. duebeni*.

1893 **Dahl** (p. 168) records *G. locusta* as one of the commonest animals in the Elbe from Hamburg down to the North Sea, and of special importance as food for fish. He adds that the species is very plentiful in the Lower Elbe in spring, practically absent in autumn, giving as an explanation of this fluctuation in numbers the statement that *G. locusta* during the summer lives on the shore under stones and among mussels (*Mytilus*) attached to woodwork and goes in winter into deep water. The animals at Hamburg, he says, live in quite fresh water with another species, *G. pulex* L., recorded by Kraepelin. He considered *locusta* and *pulex* to be very closely related, and thought it probable the 'freshwater *locusta*' might prove to be a form transitional between the two. He therefore examined specimens from the sea, and some from Altona, taking as distinguishing characters the eyes and the two rami of the 3rd uropods but no such transitional characters could be recognized. Kraepelin showed him some of his material from the Hamburg water system which he had identified as *G. pulex* L. In Dahl's opinion the specimens were definitely *G. locusta*, and he adds that Kraepelin naturally did not expect these sea forms in fresh water, and therefore had paid no attention to the small differences which distinguish them. It has already been shown (p. 581) that Kraepelin's specimens from Hamburg were *G. zaddachi*.

1892 **Chevreux et Guerne.** The two papers given in the bibliography were published at the same time, May 1892, and contain practically the same matter, viz. a description of a new species of *Gammarus* from Lake Annecy and a review of the freshwater *Gammarus* of France. The account in the *Bull. Soc. Zool.* is the more detailed, and contains figures for a comparison of the new species with *G. pulex*.

The authors mention '*G. locusta* Linn.' as a marine form, able to adapt itself to life in quite fresh water. They state (p. 141) that it is 'extrêmement commun dans la Loire, en amont de Nantes à plus de 80 kilomètres de la mer et se trouve aussi dans les rivières de la Corse et de la Provence, au voisinage de l'embouchure'. They consider that *G. locusta* has frequently been mistaken for *G. pulex* in similar conditions, and cite, in corroboration of this, Dahl's statement that the *Gammarus* of the Hamburg water supply, identified by Kraepelin as *G. pulex*, was in reality *G. locusta* 'ayant remonté l'Elbe'.

1899 **Chevreux** revised the list of the Amphipoda found on the oceanic coasts of France. It is interesting to note that he now separates the *Gammarus* of the Loire from *locusta* under the name of '*G. Duebeni* Lillj.', while giving the same locality 'au bord de la Loire, depuis Saint-Nazaire jusqu'en amont de Nantes'. The second locality mentioned, 'Prairie de Mauves', is on the river near Nantes.

But Chevreux was evidently not satisfied with his identification of the species, for after the publication of my paper in which I said that these animals were probably *G. zaddachi* (1912, p. 661), he sent me specimens from his collecting in the district, labelled '*G. Duebeni* Lillj.' and asked me to examine them and confirm the identification, if correct. Some were from 'Nantes, au bord de la Loire', some from 'Belle Île-en-Loire (eau douce)', but all, without exception, proved to be *G. zaddachi*.

1907 **Volk** gives an account of an investigation in the lower reaches of the Elbe on the effect of the sewage water from the towns of Hamburg, Altona and Wandsbek on the fauna and flora of the river. The area surveyed extended from the freshwater region of Gauert, 15 km. from the harbour of Hamburg and far above any contamination from the city drainage, down to the Third Lightship and Neuwerk Island in the estuary of the Elbe where, with high tide and the wind inshore, nearly the full salinity of the North Sea water is encountered. A chart of the district surveyed shows the tributary streams, canals and docks at Hamburg and Altona. Detailed descriptions are given of the different harbour basins, the nature of the ground, and the plant life found in them. Temperature, chemical analyses of the water, the biological collections and their quantitative distribution are also reported on.

Volk records the occurrence of '*Gammarus pulex*' in Altona Harbour, where the river receives the sewage of that town, and where it contains the typical fauna and flora found in sewage-polluted water having a low oxygen concentration. He states that, though an animal usually sensitive to contamination, it occurs here in surprising quantities. In view of the fact that the River Elbe receives the sewage of Hamburg also, the outfall being about 2 km. above Altona, he regards this occurrence of '*Gammarus pulex*' as supporting one of the general conclusions of his survey of the river which he expresses thus:[1] 'on the whole the biological relations are such that there can be no question whatever of a pollution of the Lower Elbe harmful to the fisheries, in consequence of the inflow of sewage from Hamburg, Altona, and Wandsbek.'

The '*Gammarus pulex*' collected at twenty-one stations in this Survey were sent to me for examination; all the specimens, 564 in number, proved without exception to be *G. zaddachi*, both forms, the freshwater and the saline, being represented.

1911 **Vanhöffen** (pp. 399–405) describes a collecting trip made by him in September 1911 to Pillau on the Frisches Haff for the purpose of obtaining specimens of the brackish water coelenterate *Cordylophora lacustris* Allm., the so-called 'prickly moss' of the fishermen. It forms dense masses in the eastern part of the Haff, where the water still has 1 °/₀₀ salinity. Vanhöffen found it growing plentifully in the 'Graben' (or Moat) which runs in from the Haff at Pillau, separating the old port from the new part of the town, and ending inland.

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[1] 'Alles in allem liegen die biologischen Verhältnisse so, dass von einer die Fischerei schädigenden Verunreinigung der Unterelbe durch die Sielergüsse von Hamburg, Altona und Wandsbek überhaupt keine Rede sein kann' (p. 54).

On preserving the material from the Graben, he found a rich animal life in the sediment, comprising no less than forty species, showing an interesting mixture of marine, brackish and freshwater forms, of which thirty were new records for the Frisches Haff. Amongst these thirty, he gives '*Gammarus locusta* L.', but says the specimens found were all young, judging by the small number of joints, four in the accessory flagella of the 1st antennae.

Vanhöffen sent me twenty-four of these specimens labelled '*Gammarus locusta* L.' On examination they proved to be all *G. zaddachi*, the saline form, measuring from 2 to 10 mm., the largest, strongly built and opaque, approaching the freshwater type.

1912 **Sexton** (pp. 656–65, 2 pl.). Some estuarine amphipods, taken by Klie (1913) in the old harbour at Bremerhaven, form the subject of this report. Amongst them were ten small specimens of a *Gammarus* new to science which I described as *G. zaddachi* n.sp. The largest specimen, a female 9 mm. long, was figured. I compared the animals with an estuarine form collected in the River Tamar at Plymouth in 1911, and with some specimens, labelled *G. duebeni*, from the Norman Collection in the British Museum from Suffolk, East Anglia, and found they were one and all the same species. The report was prepared, but just as it went to press, a great quantity of material became available, including, amongst others, Vanhöffen's collection from the Frisches Haff, and the estuary of the Oder; Zaddach's from Königsberg Museum (dealt with in a separate paper (1913)); and, in particular, the interesting series of samples referred to above under Kraepelin and Volk. In the reconstruction of the paper, the English samples were, through some oversight, omitted.

While this varied material was being investigated, the remarkable difference in the *appearance* of the animals was noted, and correlated with the conditions in which they lived. Those from fresh water had thick chitin in the cuticle, and long fine hairs conspicuously developed; those from brackish water, the 'saline form', were spinose with few hairs, and a thin semi-transparent cuticle. The figures illustrating the new species were taken from the latter form, the female being illustrated in this 1912 paper and the male in the next (1913). But, as so far only the 'saline' has been represented, and as so many observers have failed to recognize its identity of structure with the 'freshwater' form, I have figured both forms in detail in the present paper.

1913 **Sexton** (pp. 90–4, 1 pl.). An account is given here of the collection of *Gammarus* in the Königsberg Museum, with figures of the males of *G. locusta* (L.) and *pulex* (L.) for comparison with the male of *zaddachi*. Specimens of the true *locusta* and *pulex* from British collections and of *duebeni* sent by Prof. Sars were compared with the new species and the differences noted.

Some errors in the printing of the text and plate will be seen (e.g. two joints instead of three in the peduncle of the first antenna of *G. locusta*) which could have been avoided had the usual practice been followed of sending the proofs for correction.

1913 **Klie** here describes his study of the seasonal distribution of the crustacean fauna of the Old Harbour of Bremerhaven. He took a series of samples at regular intervals during the year March 1911 to March 1912, noting also the tides, salinity and temperatures. The water surface of the Old Harbour he says is only 7·2 hectares, with a depth at mean high water of 7·06 m. Its situation at the mouth of the Geeste, where the stream of the Weser meets it almost at right angles at the entrance, together with the narrow sluice entrance, 11 m., accounts for the relatively low average salinity of the water, 5 ‰. This is subject to constant variation, since the flushing water of the sluice at each tide causes a variation of the water level in the harbour and basin of 1 m. or more, a change which becomes very obvious through the predominance of fresh water at the ebb, and through the entrance of sea water at the flood. An increase of salinity in the height of summer each year has been recorded, as also a decrease in autumn and winter, the explanation given of this phenomenon being the lowered freshwater inflow, and the evaporation during summer. Klie quotes figures to show the variation: e.g. on 22 October 1910 the salinity was 3·35 °/oo, on the same date in 1911 it was 8·13 °/oo; on 18 December 1910 it was 1·80 °/oo, the corresponding value for the same date in 1911 being 6·32 °/oo. In the table of salinities he shows the range of the variation for one year, the lowest record being for March, 0·79 and 1·02 °/oo, temp. 7·2° and 7·1° at low tide; and the highest in September, 12·64 °/oo, temperature 17·6°, also taken at low tide. It is in conditions such as these that *G. zaddachi* flourishes, as has been observed many times, and Klie records its appearance in twelve samples, and states that it is very common and to be found in the same places throughout the year.

1915 **Tesch** (pp. 336–9), in his account of the Amphipoda collected by the 'Wodan' in the southern area of the North Sea, discusses at length two specimens, male and female, of a species of *Gammarus* from the open sea, which he could not identify with any of the known species. He refers to Hoek's work on *G. locusta* (see p. 581), in which he showed that the idea of the so-called 'variability' of the species was due to the inclusion of several different species under the one name, the 'true *locusta*' was not variable.

Tesch gives a very careful comparison of his two specimens (male 17 mm., female 15 mm.), with both the 'true' *locusta* and Hoek's '*locusta*, variety A', and shows that they agree with 'variety A' much more than with the typical form, in the following characters, viz. the proportions and the number of joints of the antennae in both sexes; the armature of the pleon segments 4–6, which differ from the typical form in not being produced dorsally in definite humps; the telson; the 3rd pair of uropods; and the proportionate length of the two rami, with their spines and hairs. Indeed, he says, that were it not for the inexplicable fact that the label of his tube of specimens gives the position of the place of capture as the open sea (St. H. 4 b., about 10 miles from the coast, and over a depth of 80 m.) and also for the knowledge that 'variety A' is recognized as a purely brackish-water form, he would unhesitatingly identify them with that form. He considers them quite distinct from *locusta* and therefore meriting a separate name.

1916 **Stephensen** (pp. 236–7, and 293) records *G. zaddachi* Sexton as new for Greenland, one specimen being taken in 1912 in a freshwater stream at Narssak Skovfjord, south Greenland. He says the specimen was small, 7 mm. long, and a little defective, but that the 3rd uropod and 4th sideplate were in excellent accordance with Sexton's figure.

1917 **Stephensen** (pp. 37–50, figs. 1–5) and

1918 **Stephensen & Ussing** (pp. 335–8, figs. 9 *a*, *b*). The *Gammarus* discussed in these papers were captured in Randers Fjord, Denmark, during an investigation undertaken with the particular object of seeing to what extent the variation in salinity influenced the fauna. The salinity range was from fresh water 0 °/oo at the head of the fjord, to 24 °/oo (bottom) and 18 °/oo (surface) at the mouth. Stephensen records five species of *Gammarus*, viz. *G. pulex* L., *G. locusta* L., *G. zaddachi* Sexton, an intermediate form between *locusta* and *zaddachi*, and *G. duebeni* Lillj.; but, he states, the specific characters are so vague that it is most probable that all the species he mentions are in reality only one species (1917, p. 37).

G. locusta L. was taken only at the mouth of the fjord in 16–24 °/oo salinity, but, in discussing its distinctive characters in order to illustrate how greatly the species can vary with the environment Stephensen described specimens of '*locusta*' from different parts of the world, from Spitzbergen, the Kara Sea, Greenland, Arctic Seas, Great Britain and east America. (The figure given, however, of a male '*locusta*' from the Kara Sea (1917, p. 40, fig. 3) is evidently the Arctic species *G. wilkitzkii* Birula (see Gurjanova, 1930, p. 588).)

Most of the specimens identified as *zaddachi* and an 'intermediate form' were taken in water of lower salinity. Stephensen, also, refers to Hoek's work on *locusta* and its varieties (1889), and unites *zaddachi* with Hoek's 'variety A', saying, however, that he considers it is not a true species but only the brackish-water form of *locusta*, and that the forms merge into each other.

Later on he re-examined this collection (see 1927, p. 120) and attributed all the specimens to *G. locusta*, acknowledging *G. zaddachi* as a distinct species.

1922 **Schäferna** (p. 96, text-fig. 6), in describing new freshwater species of *Gammarus* from rivers, brooks and pumps in the Balkan peninsula, names one, *G. spinicaudatus*, as near to *zaddachi*. He apparently accepts Stephensen's view (1917) that *zaddachi* is a variety of *locusta*, and says that his new species also is connected with *locusta* 'but more progressed than the *G. zaddachi* form'. There are not, however, sufficient distinctive characters given in the summary or in the figures to enable one to judge of the correctness of his view.

1922 **Schlienz** (p. 215) says that in studying the *Gammarus* of the Lower Elbe he found a difficulty in obtaining dependable characters on which to separate the species. He quotes Stephensen (1917) as having had the same difficulty, especially in distinguishing between *locusta* and *zaddachi*, and as having eventually reached the conclusion that the brackish-water *zaddachi* described by Sexton was only a variety of *locusta*. Schlienz goes on to state that, in his opinion, Sars's description and figures of *G. campylops* Leach, Sexton's species *zaddachi*, and his own material from the Elbe, all deal with one and the same animal, and that the one established by Leach in 1813–14.

It may be well to note here that Sars's identification of his species with Leach's *campylops* was an error. This, indeed, can be seen by comparing his figures, especially that of the 3rd uropod (1890, pl. 176, fig. 2), with Spence Bate's drawing of the same appendage from Leach's type specimen in the British Museum (1862, pl. 37, fig. 3), which was confirmed by Walker (1911) and Calman. They pointed out that Leach's types had the unmistakable short inner ramus characteristic of *G. marinus* and that Sars's animals belonged to a different species and were probably some young specimens of *locusta*. It is curious that Schlienz did not see this himself. He had the reference to Spence Bate in Sars's description, and also all the specimens of *G. zaddachi* which I had identified, in Hamburg Museum, where he could have studied them. Later, when Walker's paper came into his hands, he argued that Sars must have had a brackish-water form of *locusta* before him which was evidently *zaddachi*; that Leach's '*campylops*' became a *nomen nudum*, but the name could be retained for Sars's species; that *zaddachi* as a name must drop out, and the species should have only varietal status, and be known in future as '*Gammarus locusta* Linn. var. *G. campylops* Sars'. This matter has been dealt with here rather fully, and also in Sexton & Spooner (1940, pp. 673–5), because it is one of those mistakes in systematic work which get carried on indefinitely in the history of a species and cause so much waste of time in refuting them.

1923 **Schlienz** (pp. 429–52) in this paper gives an account of the conditions in the Elbe Estuary, such as salinity, nature of the substratum, sewage water, etc., as they affect the higher Crustacea.

Collections were made at twenty-six stations from Lauenberg, 570 km. from the river source to Cuxhaven on the Estuary, 725 km. Three species of *Gammarus* are recorded, *G. locusta* (L.), *G. duebeni* Lillj., and *G. zaddachi* Sexton, referred to throughout as *G. locusta* Linn. var. *campylops* G. O. Sars. The latter species was taken at all the stations in the area investigated, even up to the main sewer. Far from avoiding polluted waters, it occurs in almost incredible numbers below the actual harbour region of Hamburg, and is especially numerous near Mühlenberg (Blankanese), and at the Estemündung, where suitable places of refuge are afforded by the fresh and brackish-water hydroid *Cordylophora lacustris* and the two pond weeds *Potamogeton perfoliatus* and *P. pectinatus*. A table of salinities shows the occurrence of *Gammarus zaddachi* in brackish water, 0·5–18·0 °/₀₀, with the lowest mean salinity as 0·37 °/₀₀; of *duebeni* from

4·40 to 18 °/₀₀; and of *locusta*, mean salinity 18–32 °/₀₀, lowest 13·15 °/₀₀. Schlienz concludes that, while this species cannot be regarded as an indicator (Leitorganismus) for the impurity of the region in question, it still follows that, as it exists in such great numbers, it is an essential member of the living community of the mesosaprophytic zone and a considerable factor in the biological self-purification of the Elbe.

1926 **Szidat** (p. 9) enumerates the many rivers draining into the Kurisches Haff, to show the great inflow of fresh water. That the salinity of the mesohaline region is very low, he says, is demonstrated by the presence of *G. zaddachi* and *Cordylophora lacustris* on the Mole at Schwarzort, together with numerous freshwater forms. In his list of Amphipoda (p. 15) he gives '*Gammarus zaddachi* (Sexton) = *G. locusta* var. *campylops* (G. O. Sars)', as found in both the mesohaline and polyhaline regions, and as occurring abundantly at Memel.

1926 **Riech** (p. 36) gives practically the same range of salinity for *zaddachi* in the Frisches Haff. He states that it is extraordinarily numerous in the algal growth, with *Cordylophora lacustris* 'die Leitform des Brackwassers'; and that it is found in all the littoral of the mesohaline region, as well as at Pillau and other places in the polyhaline division.

1927 **Stephensen** (p. 120), in his revision of the Danish Amphipoda, acknowledges *Gammarus zaddachi* Sexton as a distinct species. He says it has never yet been taken in Denmark, and that the specimens from Randers Fjord, which he ascribed to it in 1917 (p. 41), proved on re-examination to belong without a doubt to *locusta*.

1928 **Sexton** describes a series of experiments, the aim of which was to ascertain whether or not cross-breeding could take place between closely allied species of the same genus, and, in particular, between species living in the same environment. For several years previously the material for these experiments had been in course of preparation.

Beginning in 1912 with the brackish *G. chevreuxi* it was discovered that this species was able to adapt itself to great changes of salinity and could be brought to live in any conditions from perfectly fresh water to supersaturated sea water, provided only that the change over was made gradually and sufficient time allowed for the animals to accommodate themselves to the successive increases or decreases of salinity. It was found that more time had to be allowed for the last stage—the decrease to fresh water.

Eventually six species were used, viz. *G. locusta*, marine; *G. duebeni* and *chevreuxi*, brackish; *G. pulex* fresh water; *Marinogammarus marinus*, marine littoral; and *M. stoerensis* brackish. Three stocks of each were kept going, in fresh water, in brackish and in full sea water, and throughout the work, carried on for several years through many generations, special attention was given to note what effect, if any, the changes might produce on the specific characters. The most striking point to emerge from these observations was the *inherent constancy of the specific types*. The species characters, far from being variable, were not affected in any degree by changes of temperature, salinity, food, or other conditions; growth stages could be accelerated or retarded without affecting the structure in any way; young hatched after a 10–day incubation compared with those which had taken 120 days showed no differences; and specimens brought in from the wild were identical in appearance and mated at once with those of the same species inbred in the laboratory for several generations. The characters remained stable, and were transmitted to their offspring without modification.

It was important first of all to establish the fact of this constancy of type, for in systematic work where there has always been difficulty in distinguishing between the young and immature of allied species of a genus, the suggestion has frequently been put forward that these forms might be varieties due to chance interbreeding. It was necessary therefore to investigate this question, and as some hundreds of animals were available in all the stocks, fresh, brackish and marine, a large number of reciprocal crosses were set out.

To sum up briefly: no results were obtained from any combination of *locusta*, *duebeni* and *marinus* with the other species, no matings took place, and in most of the crosses the females were eaten. Out of fifty-seven animals male *pulex* × female *chevreuxi*, five matings were recorded, no eggs laid; in the reciprocal cross, out of 148 animals, fifteen females paired and five batches of eggs were deposited, all infertile. Out of 391 animals, male *stoerensis* × female *chevreuxi*, there were only two pairings, no eggs, and most of the females eaten; in the reciprocal cross, 468 animals, twenty-nine pairings took place, eight batches of eggs were laid, all infertile. With twenty-one animals, male *stoerensis* × female *pulex*, there were five pairings, no eggs, all the females eaten; in the reciprocal cross the results were the same.

These results show that even in the most favourable and carefully prepared conditions, with each pair accustomed to the same salinity, guarded from outside attack, and provided with a plentiful supply of food, not a single cross-bred young was produced. None of the eggs laid developed, but were discarded by the females before the end of the incubatory period.

1928 **Stephensen** (pp. 275–8, fig. 58), in a key to the genus *Gammarus*, reinstates *G. zaddachi* as a species. For the principal character distinguishing it from *G. locusta* he takes the 4th sideplate; in *locusta* much deeper than broad, lower portion almost rectangular, corners rounded; in *zaddachi* not deeper than broad, inferior margin almost semicircular. Other distinguishing characters given by him are: peduncle of antenna 1 longer in proportion than in any other known species of *Gammarus*, and thickly beset with clusters of setae graduated in length; dorsal humps not very distinct; and inner ramus of uropod 3 only about three-quarters as long as outer.

He states that the species has not yet been found in Denmark and gives its distribution as recorded in Sexton (1912, 1913).

1929 **Stephensen** (pp. 138–9; text-fig. 34—figs. 241–2). This is the same account of *Gammarus* with the key and the characters distinguishing *zaddachi* from *locusta*, as that already given in 1928. He adds a reference to the occurrence of *zaddachi* in the Frisches Haff (where it is known as the 'Krabbenplage'), in such masses as to injure the fishing nets.

1930 **Gurjanova** (pp. 241–4, fig. 9) gives a redefinition and figures of *G. wilkitzkii* (Birula), one specimen of which had been taken in Ob-Jenissei Bay and described by Birula in 1897 as a variety of *G. duebeni* Lilj. Stephensen (1917, fig. 3) figured it as *G. locusta* L., and later it was suggested as the Arctic form of *G. zaddachi*. Gurjanova here distinguishes it as an independent species, and points out the characters in which it differs from other northern *Gammarus*, e.g. the shape of the head with very deep sinus; the eyes broadly reniform, relatively shorter and broader than in the other species; the numerous clusters of long hairs on the lower margins of both antennae; the shape of the gnathopod hands; and the rami of the 3rd uropods almost equal, and beset on both sides with long feathered bristles. She gives the known distribution of the species, Ob-Jenissei Bay, Nordenskjöld Sea, and Stephensen's specimen from the Kara Sea. It inhabits seas of very low salinities, and reaches a great size, up to 48 mm.

Gurjanova collected *G. zaddachi* in large numbers in the White Sea, at the mouth of the Sewernaja Dvina, in quite fresh water. The salinity in the estuary during flood-tide does not exceed 7–10 $^{\circ}/_{\circ\circ}$ and falls during the ebb to 0 $^{\circ}/_{\circ\circ}$. She considers that *G. zaddachi* and other organisms (such as *Anurea cruciformis*, *Zostera*, etc.), which are common in both the Baltic and the White Sea in the river mouths, are relict forms indicating that formerly an interchange of the faunas of these seas must have taken place.

Dr Stephensen kindly sent me three male specimens of *Gammarus wilkitzkii* from east Greenland, 65½° N., 33° W., measuring respectively 30, 44 and 45 mm. It has been suggested that this species is the Arctic form of *zaddachi*, and certainly the dense clusters of setae on the antennal peduncles give a superficial resemblance; but the points of difference are well marked, and confirm Gurjanova's view that it is a quite independent species. It can be easily distinguished from *zaddachi*, by its strongly built body, small head and eye, and by its appendages which are all longer and more slender in proportion, with the hair clusters denser and more numerous on the anterior portion of the body, less so on the posterior.

1933 **Gurjanova** (pp. 75–90), in a list of the Crustacea found in the mouths of several of the great northern rivers, in the Baltic, and in the Caspian Sea, again states that the fauna of the Northern Dwina (Sewernaja Dvina) mouth is nearer to that of the Baltic than to the fauna of the Siberian estuaries. The fact that *Mesidotea entomon entomon* and *Gammarus zaddachi* occur only in these two localities points to a former connexion between the Baltic and the White seas.

1932 **Poulsen** (pp. 1–12, figs. 1–5), when examining the invertebrates collected by the Danish Commission for Research of Sea and Fisheries, found a number of specimens of *Gammarus* in the material, captured in the waters between the Islands of Sealand, Möen, and Falster. To complete this material, collections were made in quite shallow water along the coasts.

Between the islands, the depth of the water does not usually exceed 3–4 m. and the salinity is low, generally varying between 7 and 10 °/₀₀, only exceptionally reaching 12–13 °/₀₀.

Three species of the genus were taken: *G. locusta* L. in great numbers all over the area; *G. duebeni* Liljeborg, well represented in the material; and *G. zaddachi* Sexton, found in only two localities, Stege Nor, Möen, and at Gaabense, Falster. Poulsen gives detailed descriptions of all three species, together with measurements, and figures. For the most characteristic features by which the three species can be quickly distinguished from each other, he gives: for *locusta*, the 1st joint of the 1st antenna carrying on its underside only one hair besides the distal cluster of hairs, and secondly, the distinct nodular projections on the three urosome segments: for *duebeni*, the numerous hairs on the dorsal part of the urosome; and for *zaddachi* the length of the peduncle of the 1st antenna, and the clusters of hairs on its underside; and the 'non-hairy dorsal parts of the 1st–3rd urosome segments'.

The characters hold good for comparison with the true *locusta*, but not for *duebeni*, since we now know that the freshwater form of *zaddachi* has as many if not more hairs dorsally; and similarly, not for *zaddachi* since both forms carry hairs as well as spines on the urosome and the description 'non-hairy dorsal parts' refers only to the most extreme development of the saline form. Poulsen evidently knew nothing of the difference in the development of the specific sensory armature in relation to the difference in environment (see also Crawford, 1937, p. 660).

1933 **Oldevig** (p. 199) includes *G. zaddachi* in his survey of Sweden's Amphipoda, for although, he says, it has not yet been taken in Swedish waters, it may probably be found there judging by the records of its occurrence near the southern coast of Norway. He gives its distinguishing characters and distribution, as noted by Stephensen (1928).

1933 **Palmer** (pp. 64–7) found *G. zaddachi* in large numbers, together with *G. duebeni*, in a shallow brackish-water dyke behind the sea wall at Tollesbury, Essex. They were all, as would be expected, of the saline form. The colour of the living animal is given as transparent yellowish green, varying a little in intensity, with bands of brown on the posterior margins of the body segments, antennae, peraeopods, and uropods; the oil globules on the sides of the pleon are dark purplish red. The eyes are normally black with white interommatidial pigment, but five instances out of the normal were seen among the five to six hundred specimens examined; in one, a female, the pigment was red not black, and four others had 'mosaic' eyes, i.e. some of the ommatidia red, some black, the first record of such an abnormality in the wild.

1933 **Bassindale** (pp. 297–8, 2 figs.) discovered *G. zaddachi* in the Tay Estuary during the investigations of the Water Pollution Research Board. Collections were made from the Bridge of Earn, 32 miles from the sea, near the limit of tidal waters down to Newburgh below the confluence of the Rivers Earn and Tay. The water is for the most part fresh, the salinity at Newburgh, the lowest station at which *zaddachi* was taken, at high tide reached only 3–4 °/₀₀. 199 specimens were taken, and were sent to me for identification; 198 were *G. zaddachi*, the typical freshwater form; four of the specimens had abnormal eyes, described in detail in the paper. It is curious that two reports on abnormalities in the wild should appear in the same year, this one on irregularities of eye-structure, and Palmer's, referred to above, on irregularities of the pigment.

1934 **Schellenberg** (pp. 7–13; 2 text-figs.), in his report of the Amphipoda of the Baltic, gives for the genus *Gammarus* two well-defined species, the euryhaline *G. locusta* (L.) and the brackish-water *G. duebeni* Lilj., with *G. zaddachi* Sexton named as a brackish-water variety of *locusta*.

He discusses first the distinction between *locusta* and *duebeni*. Stephensen (1929) had characterized the latter by the numerous setae on the urosome, telson, and peraeopods. This, Schellenberg points out, is not a sufficient guide, and he adds the salient distinguishing character found in the hinder peraeopods, viz. the posterior angle of the basal joint—free in all three in *duebeni*, but only in the 3rd peraeopod in *locusta*; that, together with differences in the male gnathopod hands, marks them as separate species. He comes to the conclusion that *duebeni* is sharply separated from the other two species although it has been frequently confused with *zaddachi*. All the certain records of its occurrence are concerned with brackish water of several per mille salinity, and he therefore suspects that wherever '*duebeni*' is recorded in fresh water, it is really *zaddachi* that is in question.

His next argument that *zaddachi* has no standing as an independent species, but is only the brackish-water form of *locusta*, is difficult to follow. He does not appear always to recognize the differences between the two forms of *zaddachi*, freshwater and saline, nor the distinction between them and *locusta*, and while it seems evident that he has seen these various forms, I am inclined to doubt if all his material of *locusta* is of the typical true species, or in fact if some of it may not belong to Pirlot's 'low-salinity' 'voisine de locusta' type (p. 578). This view would appear to be supported by his statements (on pp. 9, 10), where, in comparing the two species, he fails to note the differences in the shape of the 4th sideplate, and other distinctive characters such as the gnathopod hands, the development and position of the setae clusters and spines, the proportions of the rami of the 3rd uropods, etc. The most obvious distinguishing specific character to which I drew attention (1913, p. 91) he disregards, although it is an infallible guide in all the growth stages as well as in the adult animal. It is as follows: 'in *G. zaddachi*, the peduncles and flagella of both antennae carry dense clusters of long, graduated, outstanding setae: while in *G. locusta*, the upper antenna is, as in *G. pulex*, almost glabrous.' I have given figures (Pl. I fig. 1; Pl. III, fig. 19) of this character, and of the gnathopod hands of the males of the two species which should suffice to show their distinction.

Another definitive character given by me (1912, p. 662), viz. the greater length of the antennal peduncle in *zaddachi* as compared with *locusta*, Schellenberg considers as of no use for the purpose, and quotes in support of his argument the incorrect figure, of a two-jointed antennal peduncle, from my 1913 paper (pl. iv, fig. 3) but, as stated above (p. 584), I was not responsible for this absurd error in the reproduction of my drawing.

In his discussion of the distribution and salinity range of the two species he says there is overlapping, but *locusta* he rightly regards as marine and *zaddachi* as typically estuarine. He notes that *zaddachi* flourishes in the violent fluctuations of salinity such as occur in river mouths, and that it also lives well in waters of almost constant but quite low salinity, e.g. up the Elbe around Hamburg.

Finally, Schellenberg mentions various authors and their identifications, and concludes that the following, amongst others, all refer to *zaddachi*: Hoek (1889) '*G. locusta*, variety A'; Hellen (1919) '*G. duebeni*'; Schlienz (1922), '*G. locusta* Linn. var. *G. campylops* (Sars)'; Szidat (1926), '*zaddachi* Sexton = *G. locusta* var. *campylops* (G. O. Sars)': and Neuhaus (1933), '*G. duebeni*'.

1934 **Serventy** (p. 203), in his account of the marine invertebrate fauna of Scolt Head Island, Norfolk, records three *Gammarus*; *locusta* from the lower tidal levels, a member of the *Zostera* community: *marinus* from the upper levels, amongst *Fucus*: and *zaddachi*, always under estuarine conditions, widely distributed on the east coast.

1935 **Serventy** (pp. 286–94), in his observations on *Gammarus zaddachi*, gives notes on its occurrence in the River Deben in Suffolk, and its relation to the other species of the district. A diagram shows the salinity of the 12-mile long estuary, and the zones of distribution of the three principal species: *G. locusta*, inhabiting the lower part and extending to the sea outside, is the dominant form only as far as the salinity remains above 25–30 °/₀₀. *G. zaddachi* occupies the zone of decreasing salinity to the limits of the tidal influence, a distance of 5 miles. The river is very small compared with the size of the estuary, and this zone is characterized not only by a very rapid fall in the salinity gradient but also by a great fluctuation in salinity between high and low water, in one place reaching 15 °/₀₀. So that here twice daily the salinity oscillates over a range of 15 °/₀₀; an area, therefore, as Serventy points out, of peculiar rigour for marine animals, and one 'that demands a special physiological constitution, including the development to a high degree of osmo-regulatory mechanisms in its denizens'. The third species, *G. pulex*, takes the place of *zaddachi* just above the limit of saline influence in the estuary.

Serventy examined a collection made by Gurney in East Anglia, in which *zaddachi* was found plentifully represented, the specimens identified by Gurney being correctly named, those by Norman wrongly called '*duebeni*' (see also Sexton, 1912, p. 584).

Another interesting investigation was carried out by him in the Tamar, where Percival (1929, p. 93) had recorded '*G. locusta*' as occurring along the whole length of the river, from the sea to practically fresh water (0·1 °/₀₀ at high tide). As none of Percival's animals had been preserved, these statements could not be checked with the actual specimens themselves. Serventy therefore decided to collect in the brackish water section of the river where Percival had recorded *locusta*, but where, from his figures of the salinity, that species certainly could not have been expected to occur, though *zaddachi* might have been. From these localities he obtained *zaddachi* only, and there was no sign of *locusta*. Through his kindness in showing me his collection I was able to confirm the identification.

1936 **Crawford** (p. 102) refers to *G. zaddachi* as the commonest brackish-water *Gammarus* in the River Tamar and its confluents, the Tavy and Lynher, and as occurring in every stream running into these rivers. His list of localities completes Serventy's (1935) account of the distribution of the species in the Tamar.

1937 **Crawford** (pp. 647–62), in a more extended report on the estuarine fauna of the west of England and south Wales, names six species of *Gammarus* as inhabiting these regions, viz. *marinus* Leach, and *locusta* (L.) marine, the former found at a higher tidal level among *Fucus*; *chevreuxi* Sexton, *duebeni* Liljeborg, and *zaddachi* Sexton, in the brackish-water zone, though penetrating on occasions into the fresh water; and *pulex* (L.) fresh water.

Crawford describes the conditions and plant life of the various rivers investigated and gives the most important published lists of the British brackish-water Crustacea, together with certain suggested corrections of their nomenclature.

1937 **Schellenberg** (p. 482), in the course of his remarks on the systematics of the freshwater *Gammarus*, raises the problem of the cause of the numbers of different forms which yet are genetically close to each other. He asks, are we dealing with differences due to environment of such a kind that particular environments transform in a definite direction the *Gammarus* stocks which occur there? He considers the question could be answered either by experiment or through investigations as to whether with the transition from one environment to the other, one form of *Gammarus* is also transformed into the other, or whether particular forms are specially adapted for particular environments. He states, though without advancing proof, that such a step-by-step transition is seen in the case of the marine *G. locusta* going over into the estuarine *zaddachi*, and that it is most likely the gradual freshening of the waters of the river mouths which brings about the increased bristling (see Sexton, p. 587).

Schellenberg definitely concludes that environmental conditions are able to influence markedly the structure of animals, and that conditions working in the same way have developed forms similar to each other living discontinuously at places far apart.

1937 **Höfken** (pp. 116–48, 6 text-figs. and 20 tables), noting the conclusions reached by Schellenberg (1934), has attacked the problem of the Baltic species of *Gammarus* (*G. locusta*, *duebeni* and *zaddachi*) first by a statistical-variation research on a series of natural populations, and, secondly, by rearing the animals under changed environmental conditions, particularly changed salt concentrations, so as to follow up how far the development of the bristles would be influenced thereby.

He took his 'populations' from eleven places of varying salinities: from the North Sea at West Helgoland, with a salinity of about $32\,^{\circ}/_{\circ\circ}$; Kiel Harbour, of about $17\,^{\circ}/_{\circ\circ}$; Wittower Fähre on Rügen, $7{\cdot}2\,^{\circ}/_{\circ\circ}$; to Neukuhren on the Samland coast with a mean salinity of $6{\cdot}5\,^{\circ}/_{\circ\circ}$. He collected also at several places in the Schlei Estuary on a fluctuating range from 18 to $3{\cdot}6\,^{\circ}/_{\circ\circ}$, and finally from Barther Bodden at Zingst. The Zingst station differed from the others in having a fairly constant salinity, of $6\,^{\circ}/_{\circ\circ}$, and being connected with other ponds (Bodden) and the open sea only by small channels, it is cut off from the Baltic, and the interchange of water, in consequence, is slight.

In order to carry out the statistical research on the setae numbers, three characters were taken from the anterior, middle and posterior parts of the body, viz. the number of seta clusters on the 2nd joint of the peduncle of antenna 1; the number of setae in the anterior distal cluster on joint 4 of peraeopod 5 ('Pp. 7', in Höfken's numbering of the appendages); the number of spines and the number of setae on the telson; and the size of the gill on peraeopod 5 (='Pp. 7'). The whole material was divided into size groups, each 1 mm. apart; one size-group, e.g. 10 mm., included animals measuring 10–10·9 mm., the next 11–11·9 mm., and so on. The measurements were taken by stretching the animal on a millimetre scale, and counting from the tip of the head to the insertion of the telson.[1]

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[1] To my mind, an arbitrary grading of the size by such a method must lead to inaccuracy in the results. If the growth stages had been used as a standard of measurement, instead of 1 mm. increases in length, absolutely correct figures could have been obtained for each stage of the male and the female, and of the mature and the immature. It has been shown before, in *Gammarus* (Sexton, 1924), that all the individuals of a species go through a number (different for each species) of growth stages, marked off from each other by a moult, before they reach the full adult development; that each stage is characterized by an increase in size, in the number of joints of the antennae and pleopods, in the number of seta or spine clusters on the appendages, and, in the female, in the number of eggs in the broods laid in the successive breeding periods. Both sexes go through the same stages till sexual maturity is attained, after which the development is different, but, it must be noted, at any given stage, mature or immature, all the animals at that particular point are exactly alike, even to the number of setae and spines. The specific characters are *constant*, variation as such being unknown.

I have not seen any of Höfken's material, but it is evident that he clearly recognizes the distinction between the species *locusta* and *zaddachi*, though it seems doubtful whether, in the low-salinity populations, he is dealing with the true *locusta*, or what is perhaps more likely, with Pirlot's species, the 'voisine de locusta' (p. 578).

The different points of the problem which he investigated are briefly:

(1) The question whether there is a continuous transition from *locusta* to *zaddachi*, and if so, whether it is connected with the geographical distribution. The conclusion from the statistical work on nine populations is that there is no continuous transition from one to the other, and that the whole material belongs to two different types, the one weakly and the other strongly bristled, 'the *Locusta* and the *Zaddachi Types*'. There were no intermediate forms found, i.e. animals in which all the characters take up an intermediate position between the types.

(2) The next point examined was the relation between the salinity of the habitats and the distribution of the types, and here, after quoting Schellenberg's summary of the observations of previous workers and his own, and Serventy's (1935) results on the Deben, Höfken analyses his 'populations' and sums up by largely confirming Schellenberg's conclusions. These are that 'above a certain salinity, about 7 °/oo, *G. zaddachi* occurs only when strong salinity variations take place; from 7 °/oo downwards, even if these are lacking, *G. locusta* can still flourish in exceptional cases at 4 °/oo.'

The second method, breeding animals under changed salinity conditions very different from their original habitats, *locusta* in water of 6 °/oo, *zaddachi* in 18 °/oo showed a far-reaching constancy of type unaffected by the changes in environment.

Höfken summarizes his results as follows: (1) A *locusta* type and a *zaddachi* type are to be distinguished; structurally they overlap each other, but each of them is in itself independent. (2) Each of these two types occupies its own area of distribution, which, however, in places overlaps that of the other type. (3) The distribution of the two types is chiefly dependent on the mean salinity and the salinity variations. (4) The *locusta* and the *zaddachi* types are by heredity fixed types, and invariable when exposed to changes in the salinity concentration.

DESCRIPTION OF *GAMMARUS ZADDACHI*[1]

Figures have already been given of the saline form from Bremerhaven (male in 1912, female in 1913). The freshwater form is now figured for the first time, and compared with adult saline animals of the same size.

The freshwater male represented was from the Hamburg water-supply collection (p. 581), the saline male from Rauschen on the Baltic coast, Zaddach's collection. The figures of the true *G. locusta* (L.) (Pl. III), the species most often confused with *zaddachi*, are given to demonstrate their distinction.

The *body* is slender and laterally compressed, in the male larger, stronger, and more slenderly built and with stronger appendages than in the female. The chitinous cuticle varies in the two forms, being thick, strong and opaque in the freshwater, thin and semi-transparent in the brackish-water specimens. The former, also, carries a much denser supply of the characteristic long fine hairs on the antennae, peraeopods (particularly the hinder ones), pleon, 3rd uropods and telson.

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[1] The terminology which I have used in this paper is that of Stebbing ('Amphipoda Gammaridea', Vol. 21 of *Das Tierreich*), and is as follows: body, consisting of head, peraeon, and pleon: head with 1st and 2nd antennae; peraeon, with seven pairs of legs, two gnathopods, and five peraeopods; pleon segments 1–3 with three pairs of pleopods, segments 4–6 (or 'urosome') carrying three pairs of uropods and telson.

The *body colour* varies a little, much darker in some specimens than others, but is usually a pale yellowish green with bands of faint brown on the posterior margins of the body segments, the antennae, and other appendages. The females are generally darker than the males, more of a greyish green. Rathke gives the colour of his Crimean specimens as like weakly coloured joiners' glue in the male, dark grey in the female. The lateral patches of 'red oil-globules' on the first three pleon segments are very noticeable, bright red to purplish in colour. These red spots, noted by all the observers, were considered a distinguishing character of *G. locusta* at one time, whereas it is now known they are more or less developed in all the species of *Gammarus*.

Size and Sex. Compared with others of the genus, *zaddachi* is a fairly large species. There are many records of the male in favourable conditions, attaining a length of 20–22 mm.; the females are smaller, the largest about 15–17 mm. Rathke gives 10 lines for his Crimean specimens, 10½ lines for the Norwegian animals. The measurements are taken along the dorsum with the specimen straightened out on a micrometer scale, from the tip of the rostrum to the insertion of the telson.

Sexual maturity is reached at about half-growth. Zaddach found many females ovigerous during the months of May and June 1865 in Putziger Wiek, and in August 1868 he collected four small females with eggs in Danziger Bucht, the largest about 9 mm. There are many records from Britain of the species breeding from February to May.

No differentiation between the sexes can be seen in the newly hatched young, nor can it be traced till the sexual characters begin to appear several growth stages before maturity. Each growth stage ends with a moult, and by examining these moults it is possible to ascertain the state of the animal's development. This is more easily traced in the female in the brood-plates, which are attached to the ventral surface of the 2nd to the 5th segments. When fully mature they show as large thin transparent lamellae, inset on the margins with long flexible fringing hairs which interlace to form the brood-pouch.

The number of stages before sexual maturity is reached in *Gammarus* varies with the species; in *locusta*, for example, eggs are laid after the 12th moult, in *pulex* after the 10th, in *chevreuxi* after the 7th, and so on; but the development of the brood-plates appears to follow the same line in all the species observed. To take an example (Sexton, 1924, p. 345): in *G. pulex* where, owing to the thickness of the chitin, the moulted cuticle retains its firm outlines, it was possible to trace four stages of the developing brood-plates before full maturity. They first appeared in moult 7 as minute, rounded, leaf-like plates with margins entire; in moult 8 they had lengthened and increased to three times the size; in moult 9 rudimentary hairs were present on the margins; in moult 10 there was a great increase in size, and in the number of the rudimentary hairs, and the chitin was hard; in the next stage, sexual maturity was reached, the brood-plates were fully developed, with the long fringing hairs. In *G. locusta* the chitin is very thin in the young animals and tears so easily that it is difficult to find the first stages of the brood-plates; three were found, the stage of moult 10 in *locusta* corresponding with moult 7 of *pulex*; that of moult 11 with moult 9 *pulex*; and moult 12 with moult 10 *pulex* (i.e. the stage before full maturity).

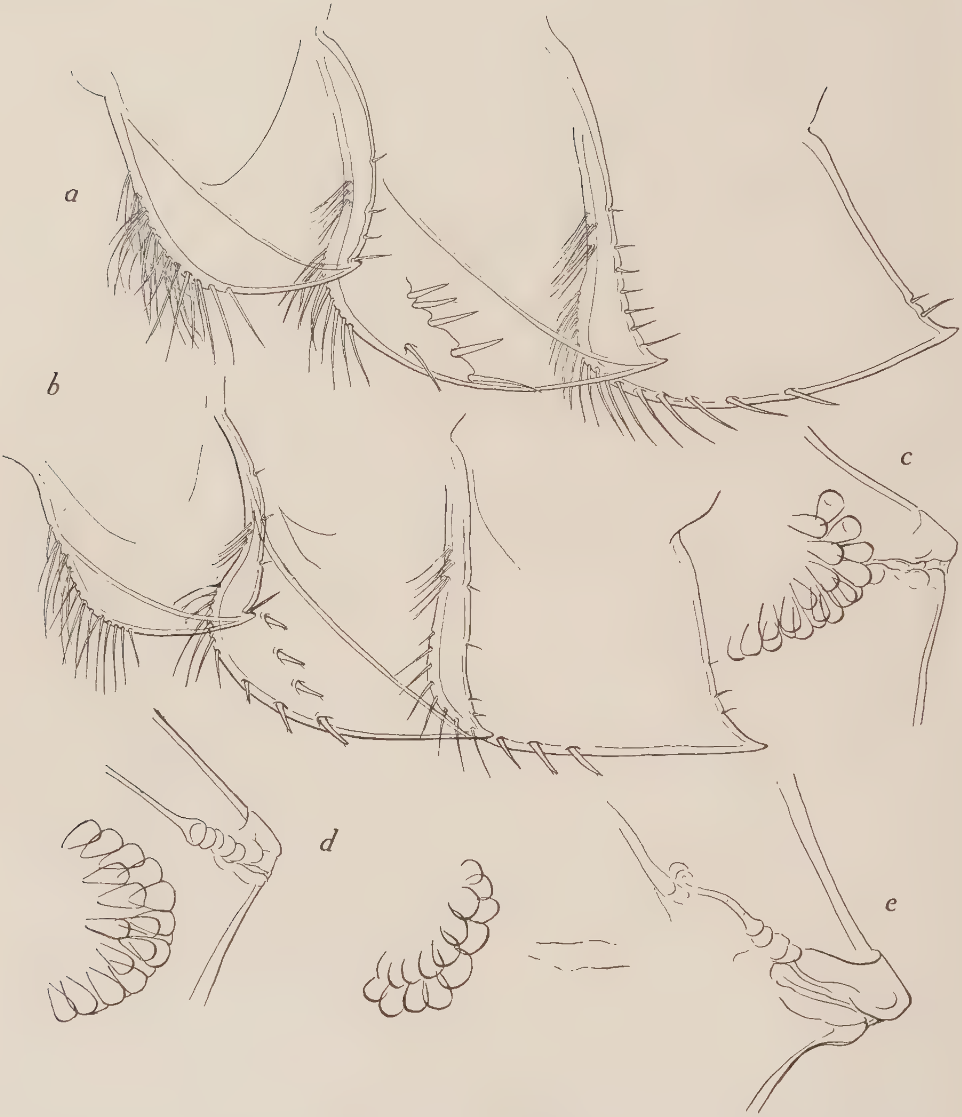
This point is emphasized here, because of the fact that certain female intersexes of *G. chevreuxi* showed an arrested development of the brood-plates, marked by a great reduction of size, and by the rudimentary condition of the fringing hairs, resembling the immature stage (e.g. in moult 9 of *pulex*). So close is the resemblance that some collectors have recorded the young immature females with partially developed lamellae as 'intersexes', but the mistake could have been avoided if the age of the specimens had been noted. In the young animal, the sensory armature is sparse, the gills and brood-plates are small, while the female intersexes are larger and much more setose than the adult normals, with the gills much bigger, and the brood-plates very small in proportion, when compared with them.

The *head* (Pl. I, fig. 1) measured along the dorsal line is about equal in length to the first two peraeon segments; in *locusta* it is definitely shorter. In both the freshwater and saline forms, the lateral lobes are obliquely truncate, with the upper angle produced, and subacute in the adults, rounded in the young and immature, as appears to be the case in the young of all the other species examined of the genus. Just below the angle and defining it is the indentation (Text-fig. 1 *c*) described by Pirlot as 'l'invagination de l'organe frontal...creusée à la face interne du lobe lateral'. In *locusta* (Text-fig. 1 *d*) the upper angle is sharply produced with the indentation small. A figure is given of this part of the lobe in *wilkitzkii* (Text-fig. 1 *e*) showing the extreme development of both the angle and the channelled indentation.

Eyes (see Pl. I, fig. 1, and Pl. III, fig. 19) large, reniform, retinal pigment black with the interommatidial accessory pigment forming a thick white reticulation (not shown in the figures, as it tends to obscure the actual size of the lenses). The ommatidia are smaller and more numerous than in *locusta*, numbering about 170–180 in the largest males. In the newly hatched young the eyes are quite round, as Zaddach (1878) was the first to point out and figure, and consist of about eight ommatidia.

Side-plates smaller than in *locusta*, about as deep as the corresponding segments; 1–4 with two or three setules inset at the rounded anterior angle. The 4th (Pl. I, figs. 7, 9), which forms one of the distinguishing characters for the species, is as broad as deep, with the posterior expansion short and rounding into the almost semicircular margin, with three or four setules inset on the hind curve. In *locusta* the posterior expansion of the 4th is very deep, with the hind margin straight, and with about thirteen setules inset on the curve. In both forms of *zaddachi* the posterior margins of the 5th, 6th, and particularly the 7th side-plates are beset with long setiform spines, most numerous in the fresh-water animals.

Pleon, segments 1–3 (Text-fig. 1 *a*, *b*): the 1st epimeral plate is deeply notched



Text-fig. 1. *G. zaddachi*, epimeral plates of pleon segments 1–3, from the males figured in Pls. I and II: *a*, the freshwater form; *b*, the saline form. × 23. *c*, *d* and *e*, upper angle of the lateral lobe of the head, right side, of *G. zaddachi*, *locusta* and *wilkitzkii* respectively, showing the opening connected with the frontal organ described by Pirlot, and the position of the eye in relation to the lobe in each species. × 75.

behind for the insertion of a seta, the 2nd and 3rd are acutely produced backwards, though not to the same degree as in *locusta*. The difference between the two forms is seen plainly in the armature, the freshwater form having many more hairs anteriorly, and fewer and more slender spines inferiorly.

Segments 4–6 (Pl. II, figs. 13, 18) slightly raised and rounded dorsally, each with the three spine groups. The number of the spines in these groups increases with age; the newly hatched young have only one spine in each group. In the adults the difference in the appearance of the two forms is more strongly marked here and in the hinder peraeopods than in any other part of the body, owing to the profusion of hairs developed in the freshwater animals. The spine formula for the saline form is usually 3 : 2 : 3; 3 : 2; 3; 2 : 2 : 2, with only a few short inconspicuous hairs between the spines, not exceeding them in length. Hoek (1889) gives the same formula for his '*locusta* var. A', and Tesch (1915) for his male specimen, and 2 : 2 : 2 on each of the three segments for the female. In the largest specimens from Rauschen the spines numbered 4 : 2 : 4; 4 : 2 : 4; and 3 : 2 : 3; and Vanhöffen's (1911) records are the same for his largest, with occasionally three instead of two in the first dorsal group. Rathke's largest Norwegian animals, 22 mm., had five in the lateral clusters of 4th and 5th segments, three in 6th.

In the freshwater form the spines are usually fewer, 2 : 2 : 2, on each of the segments, but the hairs are very numerous and very long, more than twice the length of the spines.

The *antennae* form one of the most striking distinguishing features of the species and the one most easily seen at first glance. In all the animals, saline as well as freshwater, both peduncles and the flagella carry on the under margin dense tufts or clusters of long stiff outstanding setae, some of each cluster extending far beyond the rest. The clusters in each joint are graduated in length to the distal angle, where the longest setae reach to three or four times the length of those in the proximal groups.

Antenna 1 (Pl. I, fig. 1) is not quite half the length of the body; this measurement, though formerly much used in diagnosis, cannot be considered satisfactory or reliable. The antennae seem particularly subject to injury, judging by the number of broken or regenerating flagella one finds on going through a collection. Antenna 1 is a little longer than antenna 2 in both sexes. The peduncle is unusually long for the genus, reaching in the adult to about half the length of the 5th peduncle-joint of antenna 2; in the young the peduncles of the two antennae are equal in length. The 1st joint, long and stout, is slightly longer than the 2nd in some specimens, subequal in others; the 3rd joint is rather more than half the length of the 1st; all three joints bear clusters of graduated setae on the under margin. As has just been said, it is a little difficult to give an exact number for the joints of the flagella. Zaddach has 25–35 for the primary and nine for the accessory; Hoek, for the male, 27–34, and 5–7; and for the female, 20–29 in the primary and 4–6 in the accessory. The largest males in the collections examined had 36–44 for the one and 7–9 for the other.

In addition to the long setae each joint in the primary flagellum from about the 5th carries a small stalked sensory filament on the under-surface.

There should be no mistaking this species for G. *locusta*, the characterization is so distinct. In antenna 1, for instance, the peduncle in *locusta* is noticeably shorter in proportion, extending only to the distal end of the 4th joint of the peduncle of antenna 2; both peduncle and flagellum are almost glabrous, very sparsely provided with small setae (Pl. III, fig. 19) and the accessory flagellum is much longer, 13–14-jointed in the male, with about forty-seven joints in the primary. Poulsen (1932) also pointed out that the 1st peduncle joint in *zaddachi* bore long graduated clusters of setae, while in *locusta* there is only one hair on the under-margin in addition to the distal cluster.

Antenna 2 (Pl. I, fig. 1) is stouter as well as shorter than antenna 1. The 4th joint of the peduncle is slightly shorter than the 5th, both having the characteristic graduated clusters of long setae. The flagellum is about as long as these two joints taken together, 15–19-jointed in the largest males, furnished with clusters of setae, similar to those on the peduncle, less numerous in the female than in the male. Calceolae (Pl. I, fig. 2) occur in both sexes on the proximal joints, generally on succeeding joints from about the 1st (or 2nd) to the 6th or 7th, then missing one joint, on the 8th or 9th; e.g. a male 15 mm. long with a 14-jointed flagellum, carried calceolae on the 1st to the 6th and 8th; in others of 17–19 mm. with 15-jointed flagellum the 1st–7th and 9th joints were thus provided. The largest freshwater males 20–22 mm. with 17–19 flagellum joints, have 11–12 calceolae.

Gnathopods. On comparing *zaddachi* with *locusta* it will be noted that the hands in the former are much broader in proportion to their length, with the palm less oblique and the palmar spines of the adult male different in structure and in position. In the older specimens of *zaddachi* the spines are stout, truncate and microscopically ridged on the top; whilst those which characterize *locusta*, and appear to be peculiar to it (Pl. III, figs. 21, 24), are flask-shaped, swollen at the base and constricted near the flat ridged top, with a strong central core. In *duebeni* the spines are strong, conical and pointed.

Gnathopod 1 (Pl. I, fig. 3). The hand of the male is oblong-oval, smaller than in gnathopod 2, and narrower in proportion to its length: palm and hind-margin are subequal; the palm crenulate, oblique, curving so as to end on the under-surface where the claw shuts down between the angle spine groups. One large truncate spine is situated midway on the palmar margin. The upper angle group contains six stout spines inset in pairs along the curve, the first pair consisting of one long sensory spine and one short and flat-topped, the other two pairs of short curved spines (Pl. I, fig. 4). The lower angle group also has six spines; one long sensory with two to three short curved ones inset facing the similar one on the upper side and a pair of the small bent spines below. The hind-margin carries about six rows of long stiff setae and serrated bristles.

In *locusta*, the hand of gnathopod 1 is smaller than in gnathopod 2, longer and narrower in proportion than in *zaddachi*, about twice as long as wide; the palm very oblique ending on the under-surface. One of the large peculiar flask-shaped spines is inset in the mid-margin; in the upper angle row are three graduated spines with swollen bases and a slight constriction above; in the under angle row there are one long spine and four small stout curved ones (Pl. III, figs. 20–22). The hind-margin has six rows of setae and serrated bristles.

Gnathopod 2 (Pl. I, fig. 5). The hand of the male is larger and broader than that of gnathopod 1; it is roughly triangular in shape, the hind margin about two-thirds the length of the anterior margin and subequal with the palm. There is one large truncate spine in the mid-margin separated by a gap from the four graduated stout sensory spines at the palmar angle. A slight torsion brings the palm to end on the under-surface of the hand, where the claw closes down, with a pair of small stout spines near its tip. The hind margin carries nine to ten rows of long stiff setae and curved serrated bristles.

The female has one spine midway on the palm, and a more slender and pointed claw than the male.

In *locusta* (Pl. III, figs. 23, 24) also, the hand is decidedly larger than in gnathopod 1, but is narrower in proportion to its length than in *zaddachi*. It carries three of the peculiar flask-shaped spines on the palmar margin, one midway, and the other two spaced at intervals between it and the palmar angle. The upper angle row consists of six sensory spines inset in pairs. The claw impinges on the under-surface where, with a slight torsion as in *zaddachi*, the palm ends. The hind margin has about 11–12 rows of the long setae and bristles.

The *peraeopods* show a striking difference between the freshwater and the saline forms. The figures are taken from animals typical of the extremes of the species range to show the alteration in appearance caused by the development of the hairs particularly on the 4th to the 6th joints.

In the freshwater form (Pl. I, figs. 6, 7; Pl. II, figs. 10–12) these joints carry dense clusters of very fine long hairs mixed with long slender spines; whilst at the other extreme, the 'spinose' effect of the saline form (Pl. I, figs. 8, 9; Pl. II, figs. 15–17) is produced by the great reduction in the number and size of the hairs causing the spine clusters to show up conspicuously. The spines, too, are usually shorter and stronger and often more numerous than in the freshwater form. While these two types seem constant in their own particular environment, every grade of development of the sensory armature can be found in the 'intermediate' range, in the varying salinities, but whether these grades are correlated with the particular salinity of the locality in which they are captured, or whether they move up and down with the tidal movements in the estuaries, is not known.

The peraeopods in this species differ from *locusta* in various measurements, the 4th joint, for example, being much shorter in proportion and broader; it is longer than the 6th in peraeopod 1, about equal in peraeopod 2, and always shorter in the hinder peraeopods.

COMPARISON OF THE ADULT MALES OF *GAMMARUS ZADDACHI* AND THE

	G. zaddachi Sexton, 1912	**G. locusta** (L.)
Head	As long as peraeon segs. 1 and 2 taken together. Sinus slight	Distinctly shorter than peraeon segs. 1 and 2. Sinus slight
Lateral lobes	Obliquely truncate, upper angle subacute; channelled indentation below connected with frontal organ	Angular; upper angle sharply produced
Eyes	Large, elongate, reniform, broader than in *locusta*; ommatidia smaller, 170–180. Black	Large, elongate reniform; ommatidia large, about 100. Black
Antenna 1	Peduncle long, reaching to half length of 5th ped. jt. of ant. 2; 1st and 2nd joints subequal; 3rd more than half length of 2nd. Thick clusters of graduated hairs on all 3 joints (8 on jt. 1: 8 on jt. 2; and 5 on jt. 3). Primary flagellum 38–44; accessory flagellum 7–9	Peduncle shorter, reaching distal end of 4th ped. jt., ant. 2; 1st jt. about as long as 2nd and 3rd combined; peduncle and flagella almost glabrous, only 1 seta on ped. jt. 1 under-surface. Primary flagellum 40–47, accessory flagellum 8–13 or 14
Antenna 2	Shorter than ant. 1. 4th ped. jt. slightly shorter than 5th; both carrying clusters of graduated hairs (9 on jt. 4; 10 on jt. 5). Flagellum 15 jts.; with similar hairs and calceolae	Shorter than ant. 1. 5th jt. about ⅙ longer than 4th; both with tufts of long fine hairs (9 on 4th; 9 on 5th). Flagellum 24 jts.; also with similar tufts and calceolae
Side-plates	As deep as corresponding segs. 4th as broad as deep; expansion short, inferior margin almost semicircular	Nearly twice as deep as corresponding segs. 4th much deeper than broad; lower portion deep and almost rectangular
Pleon	Epimera of segs. 2 and 3, with hind-corners subacute. Dorsal humps only slightly raised	Segs. 2 and 3, hind-corners acutely produced. Dorsal humps elevated and prominent
Spine formula	3 : 2 : 3 3 : 2 : 3 2 : 2 : 2 Numerous long fine hairs in all the groups	4 : 3–5 : 4 3 : 4–5 : 3 3 : 3 : 3 Spines and hairs shorter
Gnathopod 1	Hand smaller than in gnath. 2; oblong oval, broad in proportion to length; 1 truncate spine, mid-margin of palm; angle group of 6 spines inset in pairs. Claw closes on under-surface	Hand longer and narrower than in *zaddachi*; piriform; smaller than in gnath. 2; 1 flask-shaped spine on palmar margin; angle row of 3 spines. Claw much bent under
Gnathopod 2	Hand roughly triangular. 1 truncate spine on palmar mid-margin, separated by a gap from the angle row of 4 spines	Hand much broader and stronger than gnath. 1, narrower proportionately than in *zaddachi*. 3 flask-shaped spines on palm; angle group of 6 spines
Peraeopods	4th jt. shorter and broader in proportion than in others; hinder pps., basal jts. not much expanded; pp. 3 with hind-corner free; pps. 4 and 5 with spines inset at distal angle	Slender and elongate; hinder pps., only 3rd pp. with hind-corner free, produced to an acute angle
Uropods	Slender. Ur. 1 longest; inner ramus of ur. 3 always shorter than outer, about ¾ the length, with numerous long, feathered setae	Ur. 3 elongate, the 2 rami subequal in length, with numerous feathered setae and spines
Telson	Longer than broad; each lobe with apical group of 3 spines and hairs; subapical, 1 spine and 1 hair; subbasal, 2 spines and 1 hair	Elongate; longer than broad; apical group of 3 spines, 1 hair; subapical, 1 spine, 1 hair; subbasal, 3 spines, 2 hairs
Size	♂ 18·5–22 mm., ♀ 13–15 mm.	♂ 20–33 mm., ♀ 18–20 mm.

FOUR OTHER SPECIES WITH WHICH IT HAS MOST OFTEN BEEN CONFUSED

G. duebeni Liljeborg, 1851	**G. pulex** (L.), 1758	**G. wilkitzkii** Birula, 1897 (Arctic species)
Short; deep sinus	Very short in proportion; sinus rather deep	Small in proportion; $\frac{2}{3}$ the length of peraeon. segs. 1 and 2; very deep sinus
Vertically truncate; upper angle rounded	Vertically truncate, but less broad than *duebeni*; upper angle rounded	Upper angle acutely produced; very deep sinus
Rather smaller, reniform. Black	Small, rounded oval. Black	Small, broadly reniform, only twice as long as broad. Black
1st ped. jt. not as long as 2nd and 3rd combined, sparsely furnished with hairs. Primary flagellum 25–34; accessory flagellum short, 5–6 jtd.	1st ped. jt. not as long as 2nd and 3rd combined, almost glabrous. Primary flagellum 24–28; accessory flagellum short, 3–4 jtd.	Peduncle, 1st jt. longer and stouter than jt. 2; 3rd about $\frac{2}{3}$ as long as 2nd. Peduncle reaches to about $\frac{1}{3}$ of 5th ped. jt. ant. 2, all 3 jts. thickly beset with clusters of long hairs (8–9 on 1st jt.; 12–13 on 2nd; 7 on 3rd). Primary flagellum 54–56; accessory flagellum 8–10
Much shorter than ant. 1. 4th and 5th ped. jts. subequal. Flagellum stout, 11–15-jtd. with calceolae	Considerably shorter than ant. 1. 4th and 5th ped. jts. subequal; with numerous short straight setae like bottle-brush. Flagellum 12–13 jtd. with calceolae	A little shorter than ant. 1. 5th jt. of ped. longer and more slender than 4th; both ped. and flagellum with dense clusters of long hairs (12–13 on jt. 4; 13–14 on jt. 5). Flagellum 24–28 (broken). Calceolae seen on 21 jts.
Deeper than corresponding segs.; 4th as broad as deep; expansion deep, rounding into inferior margin	Deeper than corresponding segs., 4th as broad as deep; posterior expansion deep and subrectangular	A little deeper than corresponding segs.; 4th about as wide as deep; expansion very shallow, merging into semicircular inferior margin; angle hardly produced
Segs. 2 and 3, hind-corners little produced, acutely quadrate. Dorsal humps not much raised	Segs. 2 and 3 very slightly produced; hind-corners quadrate. Dorsal humps slightly raised	Segs. 2 and 3 not much produced. Dorsal humps not much produced, lateral clusters at a different level (farther back on segments)
3 : 2 : 3 3 : 2 : 3 3 : 2 : 3	1–2 : 2 : 1–2 1–2 : 2 : 1–2 1–2 : 2 : 1–2	3 : 2 : 3 3 : 2 : 3 2 : 2 : 2
Numerous long hairs	A few short hairs	Very numerous long straight hairs
Hand subequal in size to gnath. 2; slender, conical, pointed spine in mid-margin; palm long and very oblique; angle row of 3 spines, and a pair inset on the hind-margin	Hand about same size as in gnath. 2; piriform; palm very oblique, crenulated; stout rounded spine in palmar mid-margin, angle row of 6 spines, inset in pairs. Claw closes underneath	Hand smaller than in gnath. 2; triangularly ovate; the whole limb thickly clothed with long hairs, many dentate spines on joints; palm very oblique; 1 stout conical spine mid-margin; and angle row of 5 graduated similar spines. Claw closes on under-surface
Hand ovate, 1 slender pointed spine in mid-margin, another near angle row, almost continuous with it; angle row of 4 graduated pointed spines	Hand oblong, widening slightly at palm. Palm transverse and concave; 1 stout rounded spine in mid-margin; angle row of 3 spines	Hand very large and broad; palm almost transverse, only slightly oblique; 1 stout rounded median spine; and 5 graduated similar ones in angle row
Stouter; all 3 hinder pps. with hind corners free, produced in lobes; densely setiferous	Slender. 3rd pp. has hind-corner free and rounded; pps. 4 and 5 with expansion wide and convex; hind-corners free, though small	Slender and elongate, with fewer spines and hairs than in *zaddachi*, 5th the longest and very slender; hinder pps., basal jts. successively longer and narrower: pp. 3 hind-corner produced, subrectangular; 4th and 5th narrow to distal angle, where 2 long spines are inset
Ur. 3 inner ramus much more slender than outer, and not much more than $\frac{1}{2}$ its length; both rami densely setiferous, setae feathered	Ur. 3 long; inner ramus about $\frac{3}{4}$ the length of the outer; numerous long feathered setae	Ur. 3 inner ramus about as long as outer; both margins with long feathered setae
Shorter, broader and more spinose than in *zaddachi*; apical group of 4 spines and 7–9 hairs; subapical, 1 spine, 3 hairs; subbasal, 3 spines and 6 hairs	Rather small, and sparsely armed; apical, 2 spines, 2 hairs; subbasal, 1 spine; and 2 groups of 2 hairs each inset between	Longer and more slender in proportion; spines few, 2–3 in apical group; 1 subapical, and 1 subbasal
♂ 13–16 mm.	♂ 12–20 mm.	♂ 46–48 mm.

The basal joint in peraeopod 3 is expanded with the hind corner free and subacute; in peraeopods 4 and 5 it is narrowed distally, the hind corners not free but with one or two strong spines inset at the angle. In the older males the basal joints are long and very narrow, but in the females and immature they are always shorter and more expanded proportionately than in the adult male. The posterior margins of these joints in the saline form are inset with a few short hairs; in the freshwater forms the hairs are about three times the number and length.

The *uropods* (Pl. II, figs. 13, 14, 18). Uropod 1 is the longest; both uropod 1 and uropod 2 extend beyond the peduncle of uropod 3, and both are furnished with more spines in the saline form. In *locusta* the rami of uropod 3 are subequal in length; in *zaddachi* the inner ramus is always shorter, about three-quarters as long as the outer ramus in the adult, but the proportions vary with age and growth. In the newly hatched young, 1·5 mm., the inner ramus is not one-third the length of the outer. This character was first pointed out by Zaddach, as one of the principal features distinguishing his specimens from *locusta*. The figures show the development of the hairs and spines in the two forms.

The *telson* is about the length of the peduncle of the 3rd uropods and is longer than broad. Each lobe is armed, in the saline form, with an apical group of three spines and two or three short hairs; a subapical group of one spine and one short hair; and a subbasal group of two spines and one hair: in the freshwater form, the apical group has three spines and 9–10 long hairs; the subapical, two hairs; and the subbasal one spine and two hairs; the hairs more than twice the length of the spines (Pl. II, figs. 13, 18).

SUMMARY

Gammarus zaddachi is perhaps the most prolific and widespread of all the estuarine amphipods known to occur in northern Europe, and inhabiting, as it does, the low-salinity estuarine zone and adjacent coasts, it has come to be recognized in recent ecological work as a 'salinity indicator'.

Unfortunately, there has been constant confusion with the other common species of *Gammarus*, *G. locusta*, *pulex*, and *duebeni*, which has been greatly complicated by the difference in the appearance of *zaddachi* according as it lives in a freshwater or a saline habitat. It is shown that this difference is entirely due to the sensory equipment, the greater production of hairs in freshwater conditions, and that the structure of the two 'forms' is identical.

The history of the species has been carried back as far as I have been able to trace it (1836) with the actual specimens, described in the different papers, and the more important of these papers are discussed. It will be seen that the material examined was derived from every country of northern Europe; from Russia, the White Sea, Crimea, and the Baltic, the coasts of Scandinavia, Germany, including the Hamburg water-supply, Denmark, the Netherlands, Great Britain and Ireland, and France as far up the Loire as Nantes.

Detailed descriptions and figures of both forms of *G. zaddachi* are given; and finally, a comparison is made between the species most commonly confused with it, the Arctic species *G. wilkitzkii* being included because of a suggestion recently made that it might be, not a distinct species, but merely the Arctic form of *zaddachi*.

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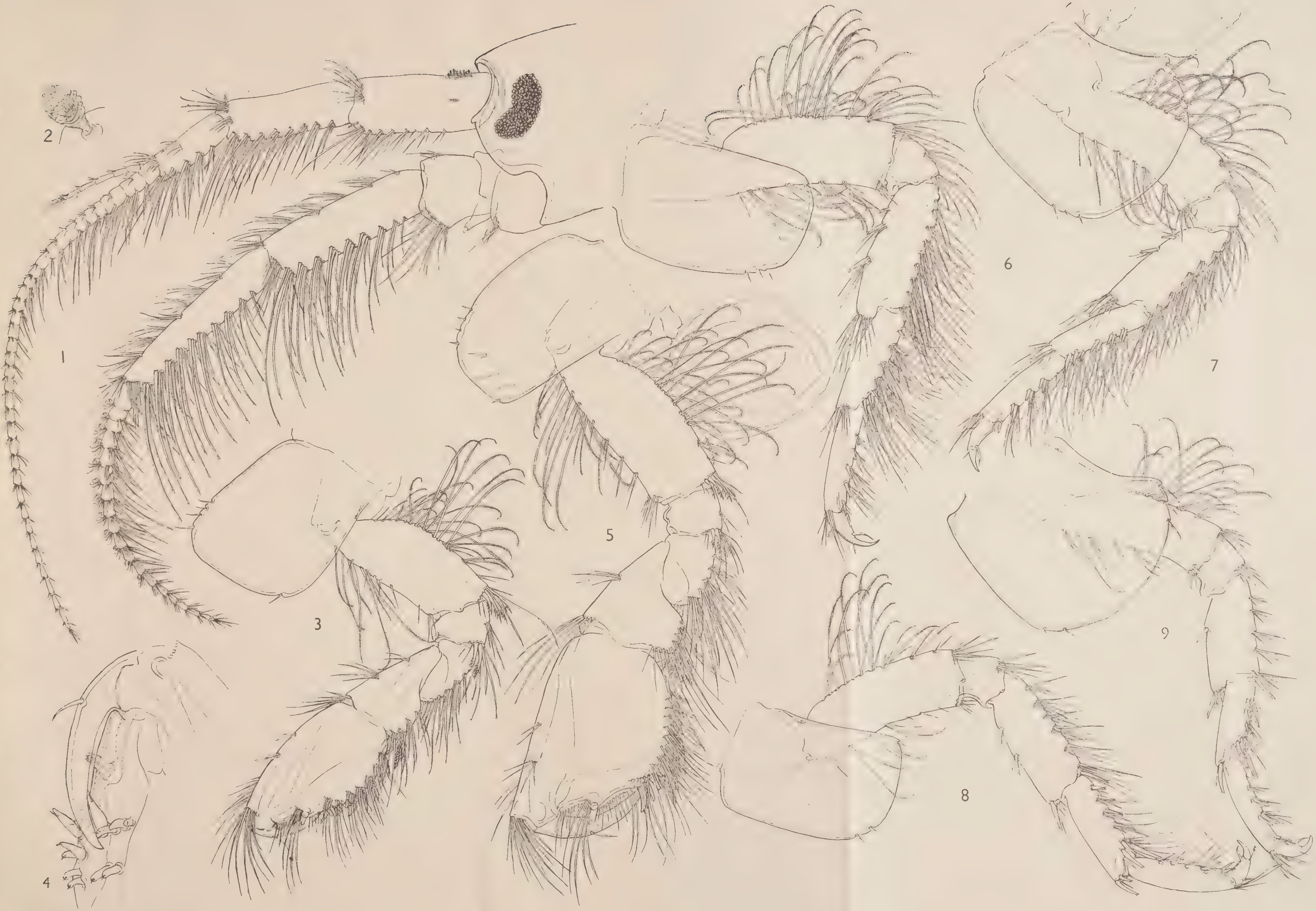

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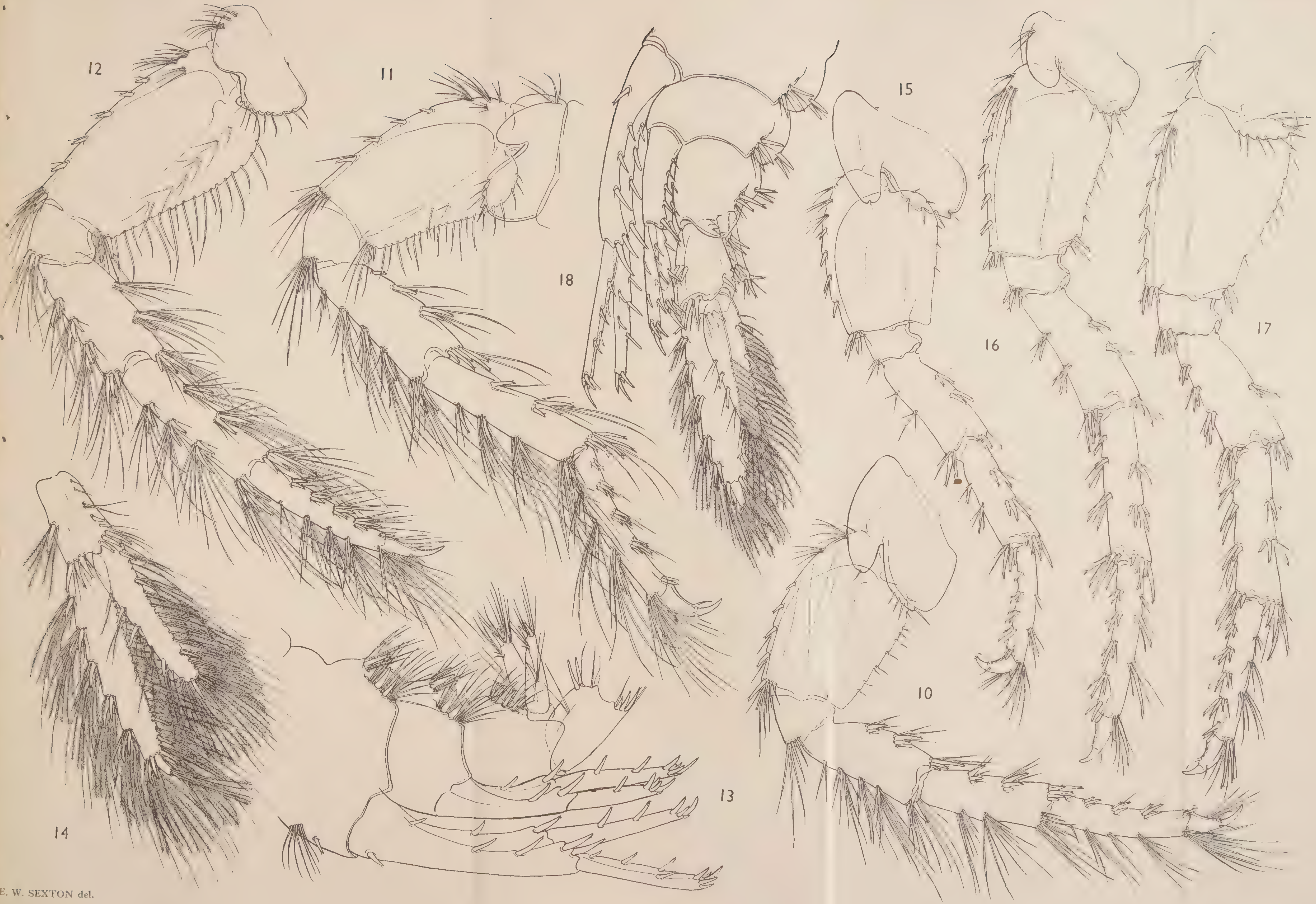
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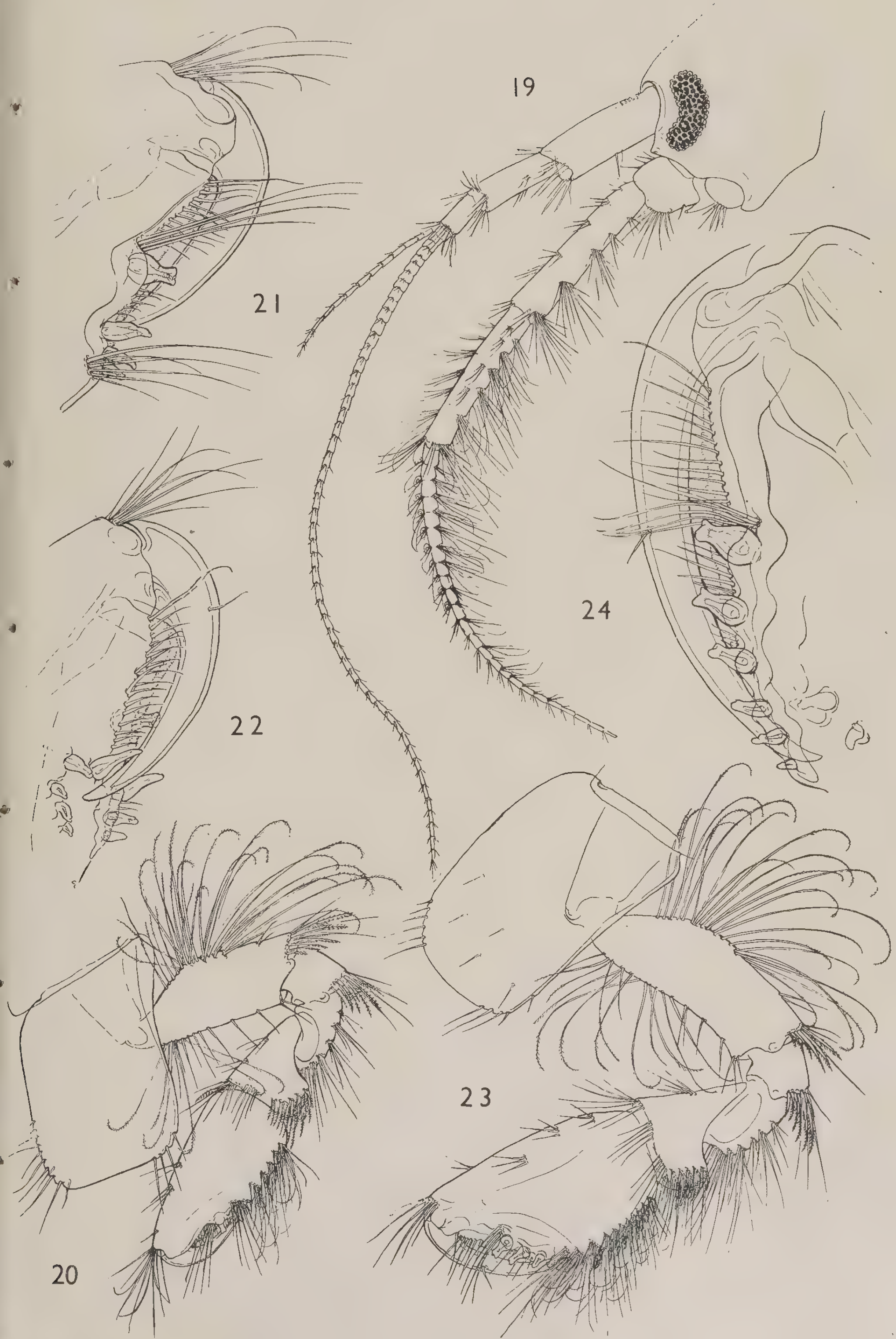
PLATE III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I have not been able to consult Tesch, 1922, *and Vanhöffen*, 1917.

EXPLANATION OF PLATES I–III

PLATE I

Adult male, *Gammarus zaddachi* Sexton

Figs. 1–7 show the freshwater form, from Hamburg Water-Supply; Figs. 8 and 9 the saline form from the Baltic.

Fig. 1. Head and antennae. × 23. (White reticulation not shown in eye.)

Fig. 2. One of the calceolae from the flagellum of antenna 2. × 140.

Fig. 3. Gnathopod 1.

Fig. 4. Distal portion of hand of gnathopod 1. Under-surface, showing the torsion of the palm, the upper and under angle groups of spines with the claw closed down between them. (The long setae are omitted for the sake of greater clearness.) ×50.

Fig. 5. Gnathopod 2, gill outlined. ×23.

Fig. 6. First peraeopod, freshwater. ×23.

Fig. 7. Second peraeopod, freshwater. ×23.

Fig. 8. First peraeopod, saline, for comparison. ×23.

Fig. 9. Second peraeopod, saline, for comparison. ×23.

PLATE II

Adult male, *Gammarus zaddachi* Sexton

Figs. 10–14 freshwater form; Figs. 15–18 saline form; from the same males as in Pl. I.

Fig. 10. Peraeopod 3. ×23.

Fig. 11. Peraeopod 4. ×23.

Fig. 12. Peraeopod 5. ×23.

Fig. 13. Pleon segments 4–6 with uropods 1 and 2, peduncle of 3rd, and telson. ×23.

Fig. 14. Uropod 3. ×23.

Fig. 15. Peraeopod 3, saline, for comparison. ×23.

Fig. 16. Peraeopod 4, saline, for comparison. ×23.

Fig. 17. Peraeopod 5, saline, for comparison. ×23.

Fig. 18. Pleon segments 4–6, showing dorsal spines, uropods and telson. ×23.

PLATE III

Adult male, *Gammarus locusta* (L.)

Magnification less than in Pls. I and II.

Fig. 19. Head and antennae; white reticulation not shown in eye. ×12.

Fig. 20. Gnathopod 1. ×12.

Fig. 21. Distal portion of hand of gnathopod 1, showing palmar spines—upper surface. ×28.

Fig. 22. Distal portion of hand of gnathopod 1, under-surface. ×28.

Fig. 23. Gnathopod 2. ×12.

Fig. 24. Distal portion of hand of gnathopod 2 with palmar spines; turned over slightly to show palmar angle more clearly; long hairs omitted. ×28.

STUDIES ON *POLYSTOMELLA* LAMARCK (FORAMINIFERA)

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(Plates IV and V and Text-figs. 1–9)

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PART I. INTRODUCTION AND METHODS, WITH AN ACCOUNT OF FEEDING

Introduction

There must be a vast number of biologists who, like myself, were taught as students a life history of *Polystomella*[1] based on the careful observations of J. J. Lister (1895, 1906) with the additions which he accepted from the work of Schaudinn (1895 *a*, p. 59; 1903, p. 500 in the *Arbeiten*[2]). To others beside myself it must have come as somewhat of a shock to realize later on the incompleteness of these observations; and most particularly the slender basis on which rests the special contribution of Schaudinn, including what might be called the crisis of the life history, the process described by him as a form of sexual reproduction.

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[1] I am not convinced that the last word has been said concerning the most acceptable name for this organism, and therefore continue to use the name so familiar in Great Britain, in spite of the fact that American and some other writers are now calling it *Elphidium* Montfort as advocated by Meek & Hayden (1864).

[2] All the page references in this paper to the works of Schaudinn refer to the collection known as the *Arbeiten*, and not to the isolated papers as they originally appeared in various periodicals.

The story of the life cycle of *Polystomella* is to be found in numerous text-books of zoology, notably in the excellent short account of the Foraminifera contributed by J. J. Lister himself in 1903 to the *Treatise of Zoology* edited by E. Ray Lankester, and still more briefly in Graham Kerr (1921). In the latter, of course, the whole story is given, including Schaudinn's addition of 1903. In Text-fig. 1 is reproduced from Arnold Lang's *Lehrbuch* (1901, p. 208) an illustration which according to its legend was sketched for this text-book by Schaudinn. This figure shows a series of stages in the life history of *Polystomella*. At A is a young megalospheric form, with its large spherical initial chamber, the megalosphere, and two others, which grows into the adult at B, still having only a single nucleus. At C the protoplasm has produced a large number of flagellate swarm spores, each provided with a nucleus and two flagella. These leave the parental shell and give rise to the microspheric form at E, with a number of nuclei and a small initial chamber, the microsphere. At F its protoplasm has crept out of the shell and divided up to form a comparatively small number of megalospheric offspring, as at A, thus completing a life history which includes a sexual (megalospheric) followed by an asexual (microspheric) generation. According to Schaudinn (1895*a*, p. 62) the alternation of the two generations is not always quite regular, for the offspring of a microspheric form may occasionally be multinucleate, and produce another asexual generation. Schaudinn claims to have observed this in three out of 4300 specimens of *Polystomella*, and points out that similar irregularity occurs more frequently in other Foraminifera, quoting *Orbitolites* and *Peneroplis*. This is believed[1] to be the case in some few Foraminifera (e.g. *Orbitolites*, *Cornuspira* and 'several other genera', Lister, 1903, p. 74: in *Rotalia* and *Cristellaria*, Lister, 1895, p. 442: also reported in other Miliolidae by Hofker, see 1930*b*, p. 28) and it may happen in *Polystomella*. But Schaudinn did not say how he made this observation—it would be a matter of special difficulty in this genus, as pointed out by Lister (1903, footnote to p. 74), and nobody has repeated it since. It might be added that once the protoplasm has left the shell it is very rarely possible to distinguish between microspheric and megalospheric individuals. I have seen in a very old emptied shell at Plymouth the initial chamber (a megalosphere) indicated by the rich growth of diatoms inside the membrane which persists after decalcification, but it is usually invisible.

Text-fig. 1 is especially interesting because it includes (at D) the only figure of a swarm spore of *Polystomella* ever published by Schaudinn. At E also is shown a dividing nucleus of which we shall have more to say later.

Two years afterwards in 1903 (p. 500) Schaudinn added his description of

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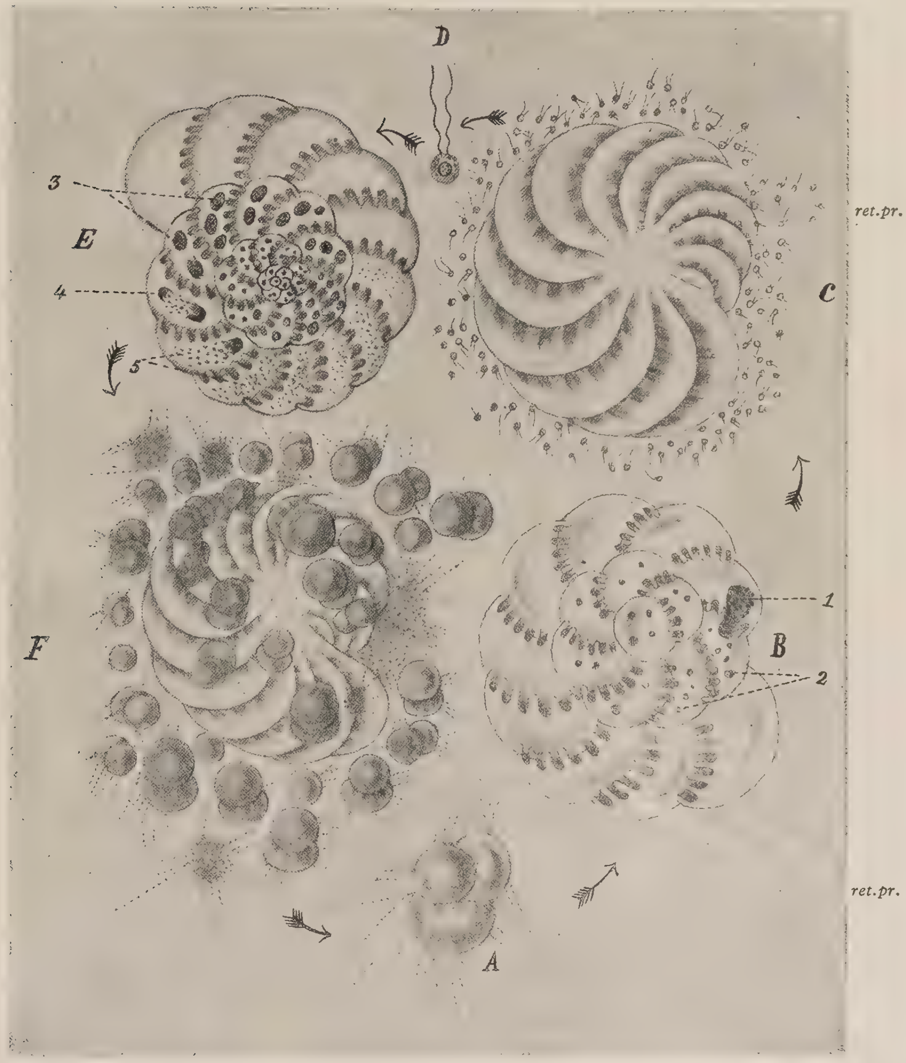
[1] But it is by no means certain in the absence of adequate measurements, etc., in the light of Føyn's analysis of *Discorbina villardeboana* (1936*b*, 1937). In this species the ranges of size of the initial chambers of megalospheric and microspheric forms overlap, so that it might, for all we know, be possible for a microspheric (asexual) parent to produce megalospheric (sexual) young having initial chambers of an equal or even of a smaller size.



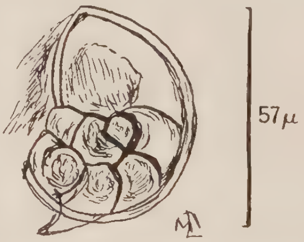
Text-fig. 1. Dimorphism and alternation of generations in *Polystomella crispa*. The arrows indicate the direction of the life cycle. A, young megalospheric individual; B, full-grown megalospheric individual, decalcified; C, megalospheric individual in the act of spore-formation, the protoplasm leaving the shell as flagellispores; D, flagellispore more highly magnified; E, microspheric individual decalcified; F, microspheric individual in the act of producing amoeboid young. 1, nucleus; 2, chromidia; 3, nuclei; 4, nucleus 'in multiple fission to produce chromidia'; 5, chromidia; *ret.pr.*, retral processes. (From Lang, after Schaudinn). After Parker and Haswell, *Textbook of Zoology*, 2nd ed., Vol. 1, p. 57. By courtesy of Messrs Macmillan and Co., London.

Three of the four guide-lines marked *ret.pr.* do not indicate the retral processes correctly. The processes are unshaded in all the figures and are projections from the concave side of each chamber.

the actual union of two swarm spores (gametes), each from a different (megalospheric) parent, to initiate the microspheric generation, which continued to develop in his culture as far as the five-chambered stage, by which time the nucleus had usually divided more than once, and then it died.

Schaudinn made extensive studies in marine aquaria which, it seems, grew up in a rather haphazard way and were, when they succeeded, probably more natural than if more elaborate and controlled methods had been employed. It is possible that by some lucky set of chances he had the good fortune to witness what so many others have striven in vain to see in more than one form of protozoon. There is, however, a disconcerting lack of corroborative evidence in the way of detail and good figures; and we find, for example, on the preceding page of the *Arbeiten* categorical statements which now appear to us almost preposterous about the 'Kopulation' of well-known intestinal flagellates. The question then arises whether he might not also have been mistaken in his interpretation of the observations under review. Further, a young microspheric *Polystomella* at the five-chambered stage might not show easily recognizable features by which it could readily be distinguished from the young of other Foraminifera. It has already been noticed, for example, that the retral processes (see Text-fig. 1 B, C) which are so characteristic of this genus are not produced by the earliest chambers in the microspheric phase (Schaudinn, 1895*a*, p. 61; Lister, 1895, p. 418). In my whole mounts of decalcified older specimens I find that one or two retral processes begin to appear on the protoplasm about the 9th–12th chamber (Schaudinn gives the 20th–25th chamber as the first of the series to show the processes; yet he refers to young *microspheric Polystomella* of 8–15 chambers without the least description of their appearance (1895*a*, p. 59)).

In April 1940, in the course of some experiments at Plymouth with the flagellate swarm spores of *Polystomella*, I found in one culture the little shell shown in Text-fig. 2. It was watched for 2 days, but unfortunately showed no sign of life. The circumstances in which it was found were such that it might quite well have been a microspheric *Polystomella* of some eight or nine chambers—a much earlier stage than any so far figured (Lister (1895) shows a young specimen of about twenty chambers in his pl. vi, fig. 7). A study of stained decalcified older specimens shows that it is quite comparable as regards its size and shape with the first eight chambers of an adult microspheric *Polystomella* (but also of some other species of Foraminifera). It was during the season of the supposed sexual reproduction, and early on the previous day three *Polystomella* had emitted their swarms of flagellate spores in a Petri dish under conditions which were as normal as possible, excepting that no food was supplied, so that one might have expected



Text-fig. 2. From a sketch by Dr M. V. Lebour of a small (? *Polystomella*) shell seen in culture. See p. 660.

to obtain some early stages of the next generation. There might have been, as can be said after making the experiments already referred to, an even greater likelihood of a very young microspheric *Polystomella* being brought in from the sea on the shell of an adult. Although these were always carefully washed, and the resulting cultures indicated that a surprisingly high degree of cleanliness was obtained, it was found that a small organism (usually a ciliate, flagellate, or small amoeba) might occasionally remain on the shell and multiply in the culture. (No other Foraminifera, as a matter of fact, had ever appeared in a long series of cultures of this kind). On the whole it does not seem justifiable, however, to identify the little shell confidently as a young *Polystomella* in our present state of ignorance of the appearance of the early stages of the microspheric phase. It is quite conceivable that it was the young of some other species with spirally arranged chambers.

On 15 March 1895, Lister wrote, 'there is no direct evidence of the conjugation of zoospores or the mode of origin of the microspheric form in a dimorphic species'; and this may perhaps still be said of *Polystomella*, although the story usually told seems very likely to be true. The only other Foraminiferan in which observed fusion of flagellate gametes has been described, leading to the development of the asexual generation, is also recorded by Schaudinn (in *Trichosphaerium*, 1899, p. 163). Again he describes the act of union of the flagellate gametes of *Gromia dujardinii* (1894*a*, p. 49; 1899, fig. on p. 163), although he did not succeed in rearing the offspring. Winter (1907, p. 19) claims to have seen the union of flagellate 'gametes' in *Peneroplis*, and le Calvez gives an account of the fusion of the 'flagellispores' in *Iridia lucida* (1938, p. 207); but neither followed the development of the zygote although the latter actually obtained a new generation in his culture. These few records constitute the only 'direct evidence of the conjugation of zoospores' in the group in spite of a great deal of work by competent workers on a number of forms of which the published life histories seem otherwise complete. This point will be further discussed.

There is, it appears, an unexpected diversity in the stages of the life history in the Foraminifera, since Myers has brilliantly demonstrated the fusion of *amoeboid* gametes in *Patellina corrugata* (1935*a*) and in *Spirillina vivipara* (1936), to give rise to the asexual generations of these two species. From his studies (1935*b*, 1936) it seems that the terms *megalospheric* and *microspheric* are not universally applicable to the sexual and asexual phases respectively even of the more typical dimorphic Foraminifera. In these two, for instance, the initial chamber (or proloculum) is not clearly divided off from the rest of the shell; and the diameter at the beginning of the spiral is actually somewhat less in the sexual than in the asexual form. For general purposes, then, it is preferable to call them by some other names such as *sporont* and *schizont*. In the present work the terms *megalospheric* and *microspheric* are retained, as they are eminently suitable in the case of *Polystomella*, though even here they have lost their widest implication.

The present studies, which it is hoped to continue when circumstances again become favourable, were undertaken with the idea of contributing something towards the demonstration of the life history of *P. crispa*, the species studied by so many generations of students in the laboratories of Great Britain. The possibility of more intensive work was provided when the Court of the University of Glasgow granted me leave of absence for the academic year 1938–9, thus enabling me to avail myself of the award of a Leverhulme Fellowship for the year. I am very glad to have this opportunity of recording my sincere gratitude to the University Court; and to the Leverhulme Trustees who have, through their Secretary Dr L. Haden Guest, M.P., so graciously expedited the work. The year was spent in the laboratory of the Marine Biological Association of the United Kingdom at Plymouth, beside the Sound where lie the famous beds of *Polystomella* whence J. J. Lister obtained much of his material, and which supply the teaching establishments of the British Isles. I am indebted to a number of benefactors who kindly bestowed on me the use of their tables in the course of the year; to a Committee of the British Association for a total period of six months; to the following Worshipful Companies of the City of London—the Clothworkers (2 months), the Fishmongers, Goldsmiths, Mercers, and Grocers (1 month each); and to the Fishmongers for another month in the Spring Vacation of 1940 when I was again at Plymouth. I offer my best thanks to Dr Stanley Kemp, F.R.S., Director of the laboratory at Plymouth, and to all those on his staff who helped me in so many ways; their friendly welcome added a great deal to the achievement and to the pleasure of the time I spent with them. Almost all my material has been collected by Mr William Searle, and I owe much to his skill and long experience for supplies in all weathers. Mr Searle continues to collect under the shadow of war in the English Channel and over Plymouth Sound. I am very grateful also to those other friends and colleagues who made it possible for me to be freed for the year from my usual routine duties, and even encouraged me to make a serious attack on a piece of work so many would have put aside as a forlorn hope. Amongst them I would especially mention the late Head of this Department, Sir John Graham Kerr, F.R.S., M.P.; Dr E. J. Allen, F.R.S., lately Director of the Plymouth Laboratory; and Mr Edward Heron-Allen, F.R.S., who gave me much material and moral support from the British Museum of Natural History. To Prof. Edward Hindle I am also grateful for some apparatus and special facilities for the continuation of the work at Glasgow, where I have in addition the ready assistance of the Director of the Marine Station at Millport (Firth of Clyde) in collecting the local material.

Material

Of the several good collecting grounds for *Polystomella* in the vicinity of Plymouth I found the material from the Drake's Island ground the cleanest and richest (Heron-Allen and Earland's Station I; see the Marine Biological Association's *Plymouth Marine Fauna*, 2nd edition, p. 34). All the catches brought in for me during the year I was at Plymouth came from there. After the middle of March 1939 the '*Gammarus*' went out specially and brought the material straight back early in the morning. Since this ground ceased to be available in September 1939 my *Polystomella* have been collected at the station known as 'White Patch', nearer the east end of the Breakwater.

A widespread and much the most plentiful species in this neighbourhood is *P. crispa* L., and, without claiming any special qualifications as a systematist on the shells of the Foraminifera, I believe that all my work has been done with it. This is probably only of importance in the experiments with the flagellate spores, and in order to be reassured particularly at this point I have called in the skilled assistance of Mr Arthur Earland, F.R.M.S. I shall refer to his opinions in the course of these studies; and gladly take this opportunity of thanking him for his ready help.

Fixation of Material

Experience had shown that the usual methods of fixation give very poor results with *Polystomella*. This is presumably due, at least in part, to the heavy calcareous shell of complicated form, inside which most of the massive protoplasm is only freely accessible through very small openings until the calcium carbonate is dissolved away; this takes quite an appreciable time—several hours if the reaction is not to go on with such violence as to break the structure up. So that apart from the old antithesis between good fixation of nucleus and of cytoplasm respectively there are further difficulties to be overcome, and these increase with the size of the specimen to be fixed. One such difficulty is only too obvious after fixation: a more or less violent protoplasmic movement is apt to occur, the cytoplasm surging from one chamber to the next, the nuclei often becoming drawn through the foramina between the chambers and fixed there in a great variety of bizarre shapes—even though their inner structure may be quite well preserved. That these distorted nuclei were not all caught in the act of passing through from chamber to chamber, as has been suggested, is clear because of their constant relative incidence according to the method employed. It may be that the difficulty in obtaining an undisturbed fixation is also partly due to an extreme sensitivity and very quick movement of the protoplasm. A living *Polystomella* may move very swiftly, the long fine pseudopodia being very active outside the shell, while the cytoplasm in the chambers may be seen at times to be in rapid circulation.

A great many trials were made with most of the usual fixatives and variants of them, including corrosive acetic mixtures, with and without alcohol, Zenker, Susa, trichloracetic acid, Bouin, with and without urea and chromic acid, Gilson, Carnoy, Flemming and other osmic acid methods. It is not proposed to describe them all in detail. None of them gave perfectly reliable results with large *Polystomella*, and each has its fairly constant defects; but a very great improvement has been found possible, at any rate for some stages of the life history.

Cold fixatives, i.e. up to room temperatures, are as a rule hopeless for the nuclei. After such the nuclei often show no internal structure at all, being fixed only on the surface at one side, thus producing a figure more or less like that of an open empty tow-net. The fixation is much improved by heat, especially if the degree is carefully selected to reduce protoplasmic streaming to a minimum. Hot fixatives, on the other hand, have the disadvantage that their application dislodges more of the foraminal plugs ('bouchons' of le Calvez, 1938, p. 236, see p. 625 and Text-figs. 6, 7), sometimes causing them to be found lying loose in the cytoplasm of the terminal chambers, and sometimes to be thrown right out of the creature.

Corrosive acetic mixtures have been the classical fixatives for Foraminifera ever since both Schaudinn and Lister chose them for this purpose, using them 'warm' (Lister, 1895, p. 414). Lister's figures show the limitations of the method, as do his remarks in the text about protoplasmic disturbances (p. 416), the radial arrangement of the cytoplasm round about the nuclei (p. 418), and the absence of a nuclear membrane (p. 419). After trying many modifications I prefer a 6 % solution of corrosive sublimate in sea water, to which I add 5 % of glacial acetic acid, used at a temperature of 60–65° C. The specimens are put with a pipette into the fixative previously heated in a closed dish on a water bath, and after 5–10 min. the dish is removed to the bench and left to cool for an hour or so. Decalcification proceeds, and the *Polystomella* may be lifted towards the surface by the bubbles coming off the shells. Inspection shows, however, that they do not actually rise to the surface because as soon as the bubble touches the surface film it is stopped, even if it is not discharged; there is no risk of the specimen drying up as it hangs below in the liquid, and it is perhaps better to leave these apparently floating specimens alone. If decalcification is not completed in the fixative it is usually finished off in 70 % alcohol with 3 % of nitric acid, after thorough washing in 30, 50 and 70 % alcohols in succession, a few drops of iodine solution being added to the last. Fixation by this method is not free from the imperfections already mentioned, though they are reduced as far as possible. The result is fairly good and of course excellent for staining. I do not find the addition of alcohol any advantage, nor the increase of the acetic acid to 25 %. Higher and lower temperatures give worse results. I have not been so successful with the method which gave Myers (1935 *a*) such good preparations of *Patellina*[1]—although I found that his Schaudinn's solution (corrosive acetic with alcohol, containing 25 % acetic acid instead of the usual 4–5 %) gives slightly better results at higher temperatures (80–90° C.) than at 60–65° C.; my less drastic mixture has the advantage also at the higher temperature.

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[1] I had the great pleasure of seeing some of Dr Myers's fine preparations at Plymouth, as he and Mrs Myers arrived from California quite unexpectedly in the summer of 1938. They decided to work at *Polystomella* while they were at Plymouth, and we agreed to continue our separate researches, which lay along such different lines, quite independently, although for a time under the same roof. It is a pity that our records cover the same year—but our coincidence was purely fortuitous. I greatly appreciated their offer to show me their special paraffin embedding bath before its publication—part of the technique they had evolved in their long experience with the Foraminifera, and which enables them to deal with the astronomical numbers of *Polystomella* on which their results are based. I found the apparatus very useful in sectioning my much smaller sets of specimens, although for special individuals, of course, solitary embedding is to be preferred.

Better effects were obtained with a modified Zenker's solution, viz. sea water, 100 c.c.; corrosive sublimate, 5 g.; potassium bichromate, 2·5 g.; with 5 % glacial acetic acid added immediately before use (and omitting the usual sodium sulphate).[1]

This solution was best used at 40–50° C. for about 10 min. and then left for about 2 hr. on the bench to cool. Care must be taken afterwards to wash very thoroughly in water to avoid a precipitate in alcohol. The excess sublimate is removed with iodine in the usual way. On the whole the cytoplasm and nuclei look less disturbed after this fixation. Most of the nuclei, especially in the megalospheric form, are more often nicely rounded, showing a distinct membrane. Radiations in the surrounding cytoplasm are usually absent at this temperature, but in a few cases there is a small shrinkage space about the nucleus.

The only other useful results with large *Polystomella* were obtained with osmic acid methods. 'Flemming' was used by Lister with some success, though he barely mentions it in his published work; and from his results it was probably used warm, though he does not say so. Various modifications of these solutions were therefore given a thorough trial, the acetic acid always being added at the time of using.[2] They all produced much the same result.

In the cold, i.e. at room temperature (about 14° C.) or using ice (about 2–3° C.), after several hours' application, the cytoplasm was excellently fixed, the fatty constituents being preserved and giving it the crowded appearance it has in life;[3] but the nuclei are very bad, often showing the townet shape and no internal structure at all. There was unfortunately a tendency to violent surging of the protoplasm from one chamber to the next, no doubt rendered very conspicuous by the subsequent good fixation. When the solutions were used hot (35–40° C.) the nuclei were as usual greatly improved, but although a few good ones were seen, on the whole they are wrinkled and not so well fixed as with Zenker or corrosive acetic. Once or twice I noticed a slight exudation of protoplasm from the shell similar to that which occurs when a living *Polystomella* is put into fresh water (see p. 620, footnote 4). It was seen after the use of strong Flemming, made up in fresh water and used at room temperatures or higher.

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[1] After an interesting talk with Mr J. Z. Young on the speed of ions and fixation (Young, 1935) I doubt whether the sea water is any improvement with corrosive acetic or Zenker—but with the latter at least its use saves trouble.

[2] 'Strong', 'weak', and an intermediate solution of Meves (see Gatenby & Cowdry, p. 377) were tried; also other variations with more or less chromic or osmic acids, and Føyn's mixture made up in sea water (1936*a*, p. 275). Various times (1½–21 hr.) seemed alike in their effect.

[3] It is hoped to analyse the cytoplasmic inclusions in a later part of these studies.

When desirable before staining it was found quite satisfactory to bleach in hydrogen peroxide solutions either whole *Polystomella* or sections blackened by fixation in osmic acid.

It was noticed that with the osmic solutions the nuclei were not pulled through the foramina, and some experiments were made with the object of attempting to combine the advantages of these solutions with those of the corrosive acetic mixtures with which the derangement of the nuclei is a most serious drawback. The *Polystomella* were allowed to crawl on well-cleaned microscope slides; and when their pseudopodia were well extended the slide was cautiously inverted over osmic vapour. After the desired exposure the slides could be transferred face downwards to other reagents, or the *Polystomella* washed off with sea water and immediately subjected to further treatment. (Incidentally this method was used to make whole preparations of *Polystomella* with extended pseudopodia, with or without subsequent treatment to dissolve the shell or to improve the fixation of its contents.) In the cold the best results were obtained with an exposure to the vapour for 10 min., followed by immersion in 6 % corrosive sublimate with 5 % acetic acid, in sea water. But as usual there was a great improvement when the *Polystomella*, after exposure to osmic vapour, was washed into the corrosive acetic at 60–65° C. With the osmic vapour the canal system which runs through the shell (see Lister, 1903, p. 65) is particularly well shown up, being well fixed and slightly darkened; also the cytoplasm, particularly in the outer chambers. Sometimes the whole effect, including that on the nuclei, was very good, notably in the case of one or two large microspheric *Polystomella*, which are usually very difficult subjects. The protoplasmic disturbances seem to be reduced, more foraminal plugs remain in situ, but on the whole the fixation of the nuclear structure is not improved by osmication before hot corrosive acetic and indeed may be rendered worse. It seems likely that the exposure is rarely just right, and it may be unequal in different parts of a single large specimen.

The rather complicated mixture known as Heidenhain's 'Susa',[1] used at 40° C., produced some good nuclei, but tended to give a washed out general appearance to the preparation. Small *Polystomella*, below twenty chambers or so in a megalospheric specimen, or thirty in a microspheric one, are more easily fixed. With these the best results have on the whole been obtained with 'Susa' at 40° C.

If for any reason it is not possible to use the fixative hot, a preliminary treatment with osmic vapour may be advised before corrosive acetic. Next to corrosive acetic with or without osmication, perhaps Zenker gives the best results in the cold.

Most of my material was fixed in Zenker or corrosive acetic, both used hot, excepting small specimens for which I often used 'Susa', also hot. The fixation was quite satisfactory as regards staining properties, which were still good after a year or more in 70% alcohol when it was unfortunately necessary to keep the specimens unstained for so long. An interval not longer than a month was aimed at in the case of Feulgen's stain, however, and when this was exceeded the fact will be noted. For staining whole mounts borax carmine, well differentiated, gives good results; and picrocarmine even better, especially for osmic acid preparations. Ehrlich's haematoxylin may be used for very small specimens only. Sections are generally stained with Ehrlich's haematoxylin and eosin. Mann's stain gives very beautiful results with thin sections (8μ), the foraminal plugs coming out yellow, while the chromatophores, to which the colour of the living animal is due, are pink.

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[1] See Brontë Gatenby (1937, p. 74). Water, 80 c.c.; corrosive sublimate, 4–5 g.; sodium chloride, 0·5 g.; trichloracetic acid, 2 g.; glacial acetic acid, 4 c.c.; formol, 20 c.c.; or Romeis's simplification of the same (Romeis, 1928, p. 80), saturated aqueous corrosive sublimate, 25 c.c.; 5 % trichloracetic acid, 20 c.c.; formalin, 5 c.c. The instruction is to proceed direct from these fixatives to several changes of 80–90 % alcohol.

Cultures

It was obviously desirable to have *Polystomella* growing in cultures, and this has been achieved with a fair measure of success. Further experiments are contemplated with a view to improving the results already obtained. *Polystomella* lives, grows, apparently remains in good condition, and proceeds with its life history as long as it is kept in a pasture of flourishing diatoms. To this end I used the medium known as 'Føyn's Erdschreiber'—sterile sea water + salts to which an earth extract is added—with excellent results.[1] I did not attempt to free my cultures from bacteria, nor from small flagellates, both of which might have been useful in the feeding of the Foraminifera. The diatoms were grown in Petri dishes, and small numbers of *Polystomella* (up to four or five adults or about twenty young) lived here as long as the diatoms were plentiful and of a good healthy brown colour—usually 3 or 4 weeks. Larger numbers or growing up families were kept in cultures grown in larger receptacles, glass pneumatic troughs measuring 9–10 in. in diameter and 4–5 in. high being found very suitable for cultures 2–3 in. deep; and flat-sided glass museum jars with at least one polished side for purposes of observation with a horizontal microscope. The diatoms were usually grown at room temperatures, excepting in the hottest summer weather at Plymouth, but the *Polystomella* cultures were kept at various temperatures as will be noted in the course of this paper. A moderate light suits most diatoms; on dark winter days extra light was sometimes supplied from ordinary electric lamps overhead, and in the summer at Plymouth the light close to the window, even though this faced north, seemed too bright and the cultures were moved farther away. The rate of growth of the diatoms naturally varies with conditions of light, temperature, the density of the sowing and the state of the diatoms themselves, and it is a matter of experiment to determine the time necessary to produce an adequate feeding ground. Sometimes the life of a culture was prolonged by renewing the culture medium in the dish.

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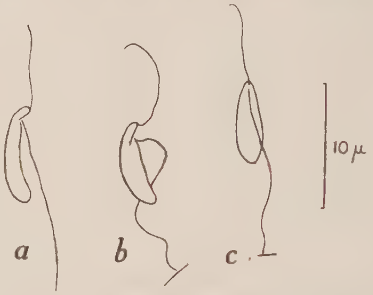
[1] I am very glad to take this opportunity of expressing my best thanks to Dr Fabius Gross, Lecturer in Zoology in the University of Edinburgh, for so kindly showing me his technique for the culture of planktonic diatoms, both at Plymouth in 1936 and in Edinburgh in 1938. See Gross (1937), where he gives the recipe for the culture medium which I also used for bottom diatoms, viz. sterile sea water, 1000 c.c.; soil extract, 50 c.c.; $NaNO_3$, 0·1 g.; $Na_2HPO_4 . 12H_2O$, 0·02 g.

Several different diatoms were cultivated, including one or two planktonic forms, but the most suitable type was found to be the motile bottom-living Pennatae which would spread evenly over the surfaces covered by the culture medium, preferably rising up the sides of the vessel as well as covering the floor. I had the good fortune to catch an ideal form in November 1938 by crushing in a watch-glass of Erdschreiber one of the empty *Polystomella* shells from the sea, which at Plymouth sometimes contain crowded and almost pure cultures of a single diatom of this kind, often along with nematodes and various protozoa. From this source was obtained without further purification a culture containing a single species of naviculoid diatom, which was identified in a subculture of some months later as *Navicula mutica* Kutz. var. *Cohnii* (Hilse) van Huerck.[1] There was also one easily recognized flagellate which persisted unchanged, though its numbers might fluctuate, throughout the year in which the cultures were kept going. It was 8–10μ long, with a very rapid dancing motion, and no tendency to amoeboid action, keeping its shape unchanged except for slight movements of the mobile anterior end. The results of a somewhat cursory examination are shown in Text-fig. 3 and Pl. V, fig. 1, from which it is clear that our flagellate may be included in the genus *Bodo*. It does not agree very closely with the description of any of the well-known species—from fresh-water habitats. Its shape and activity place it nearest to *B. celer* Klebs (1892, p. 313). No contractile vacuole was observed, nor any attempt at encystation, but this is not surprising in a marine flagellate. Neither was it seen to feed by sucking out other protozoa, but there was small opportunity of this in my cultures, unless it were extensively cannibalistic. I shall refer to it simply as *Bodo* sp.



Text-fig. 3. *Bodo* sp. living, from culture of *Navicula mutica*. *a*, swimming freely, *b* and *c*, slightly shortened and thickened as when anchored by long trailing flagellum, which also shows a tendency to wind spirally round the body.

The *Navicula*, on the other hand, unfortunately underwent a progressive deterioration in the series of subcultures. At first (December 1938) it was a plump oily diatom of the usual naviculoid shape, about 20μ in length and about 6μ wide in the middle, of a rich brown colour. It had considerable motility which enabled it to spread evenly over surfaces. The motility decreased with age, and the diatom became enclosed in a thick (? mucous) coat to which bacteria adhered. When the colour of the growth changed to a yellower shade it was due for replacement as a feeding ground for *Polystomella*.[1] The most suitable temperature seemed to be about 55–60° F. (14–16° C.); at higher temperatures the growth came faster, but it was paler; lower temperatures gave a slower growth but of a richer brown. In the winter with a moderately thick sowing a Petri-dish culture at Plymouth would be ready for use after about a month, while the old one would need replacing—in the spring and early summer the plates developed in half this time or less. Almost from the beginning it was noticed that there was an admixture of shorter cells in the cultures—down to 10μ or so in length—which tended to grow in short chains or little heaps; and at times these would predominate in a patch of diatoms, the general growth habit at the same time becoming rough and not attached to the substratum. I believe, however, it was a pure culture, and that I obtained at various times a smooth growth of 18–20μ diatoms from a rough growth of short ones and vice versa. This went on throughout the summer, but I believe the original size of the longest diatoms was not regained after a while. In August 1939 it is noted at 16μ; by the following February it did not seem to exceed 10–12μ. In the spring of 1940 the cultures got much worse after a very dark winter in Glasgow, finally producing only very slowly a few cuboidal cells 5–6μ across, in little heaps, and the strain was abandoned. The shape of the cells had degenerated until they could at times not be recognized as naviculoid diatoms at all.

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[1] I am indebted to Mr R. Ross of the British Museum of Natural History for this identification. He says, however: 'I am not really satisfied about the *Navicula*, and it would be better to query the identification for the present.' The uncertainty is probably to be explained by the gradually increasing abnormality in the subcultures described in the text.

It was very interesting to see in February and March 1940 the *Navicula mutica* cultures which Mr D. P. Wilson had very kindly maintained for me at a lower temperature after I left Plymouth in September 1939. They appeared also to have lost their original size, but had not then declined so far as my diatoms which had been cultivated for use and much more vigorously; by the autumn, however, they seemed almost as bad as mine were in the spring.

Unfortunately, time did not allow of a closer investigation of the diatom cultures I was growing as food. I thought that the dying out might be due to an increasing unsatisfactoriness of the periodic auxospore phases owing to some imperfection in the conditions of cultivation.

The only other diatom I cultivated at all extensively for use at Plymouth was *Synedra tabulata* (Ag.) Kütz.[2] This was a beautiful golden brown form 40–50μ long, not a naviculoid and not motile. Therefore it did not spread well in the cultures but grew in heaps lying loosely here and there on the floor of the dish, excepting when it was spread about by browsing *Polystomella*. It was cultivated in Erdschreiber from November 1938 to July 1939, when it was given up since it had been showing irregularities for some time.

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[1] As M. Schultze remarks (1854, pp. 20, 24), the colour of *Polystomella* depends on its state of nourishment, and agreed as far as his chemical investigation went with that of the diatoms on which it largely fed (the diatomin of Nägeli).

[2] Also identified by Mr R. Ross. This diatom appeared in a culture of *Enteromorpha* sp. (kindly named for me by Miss E. Stanbury, of the Technical College, Plymouth) which came from an aquarium taken by Dr Gross from Plymouth to Edinburgh some months earlier.

Latterly one or two other bottom-growing diatoms, some from the *Corallina* shore pools at Millport[1] and some at least naviculoid, have been tried at Glasgow, and have seemed more or less satisfactory as food for *Polystomella*; but I have not come across any which gives such a beautiful even growth as did *Navicula mutica* at its best. These cultures of 1939, which could be grown to any required density on any clean glass surface, gave me the opportunity for many hours of fascinating observation of living *Polystomella* going about their business, the results of which I hope to record in these studies. As Max Schultze wrote of his work in 1854, p. vii: 'Der Hauptzweck dieser Arbeit ist, durch Mittheilung treuer Beobachtungen über den Bau und die Lebenserscheinungen der beschalten Rhizopoden der Meere unsere bisher so lückenhafte Kenntniss derselben zu ergänzen.'

The *Polystomella* were taken from a D-netting which was given a preliminary washing at sea. As soon as the catch arrived in the laboratory it was put through a sieve to remove large particles, and well washed with 'outside sea water'[2] in a large flat white enamel dish until as much as possible of the remaining mud, etc., was removed. After a few hours the *Polystomella* had crept up on to the surface of the catch and some up the sides of the dish, whence they could be easily picked off with a pipette. The shells chosen for culture were again cleaned; first with a small brush[3] under a binocular, and then washed with a pipette in several changes of filtered outside sea water.[4] After this treatment it was found as a rule that no other protozoa were introduced into the cultures, the protoplasm of the *Polystomella* being withdrawn inside the shell so that nothing appeared to stick to it so long as it was kept agitated. If, however, it is left still for a few minutes the protoplasm emerges and pseudopodia are put out almost at once.

It was sometimes desirable to be able to recognize individual *Polystomella* without having each one isolated in a culture by itself; and also to know when growth had taken place, this being most clearly indicated by the formation of additional chambers to the shell. Since the shell is not normally external to the cytoplasm it seemed better not to risk an attempt to mark the shell itself in any way. Very often individuals could be put together which showed some marked difference—a set of small chambers due to irregular growth, or a protuberance, for example, would serve as an identification mark, or as a landmark in a growing shell. But best of all were clear signs in the form of stalked egg cases or cocoons which occur not infrequently attached to the *Polystomella* shells at Plymouth.[1] These are almost always empty as shown in Text-fig. 4 *b*, but usually remain firmly fixed by the basal plate of the stalk.

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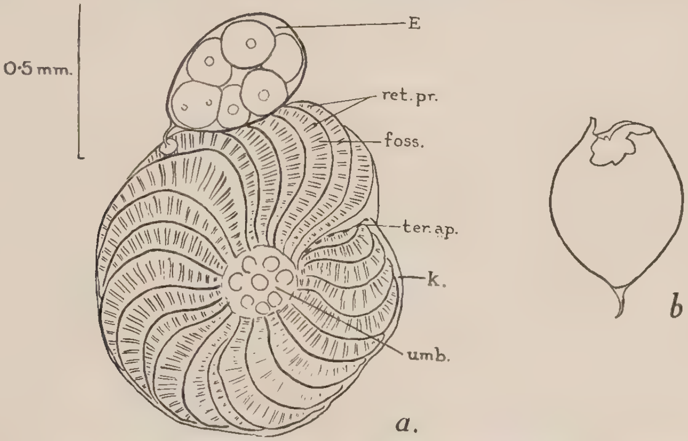
[1] These pools support a small population of living Foraminifera of several species, including some small *Polystomella*. Mr Earland has examined a number of these and assures me they are all *P. crispa* of a small size (under 1 mm.) and often showing spines at the shell periphery, or with a tendency to make very thin shells with rather bloated chambers and no keel—probably ill-developed forms surviving under hard conditions.

[2] Brought in from outside the Breakwater in carboys (cf. Lister, 1903, pp. 69, 70).

[3] A red sable brush no. 00, for miniatures, is very suitable. It has a fine point, can be sterilized in boiling water, and stands hard treatment well.

[4] Fresh water may not be used as it causes the protoplasm to swell and ooze out at the terminal apertures and from the fossettes (see Pl. IV). This swelling in fresh water also occurs in other Foraminifera.

These cocoons were found still occupied in April and May 1939, from the Drake's Island ground; in October 1940 and in June 1941 from 'White Patch'. They will continue with their development and hatch in sea water or diatom cultures in Petri dishes in the laboratory. The earliest cocoon contained 6–8 rounded objects (see Text-fig. 4 *a*) in which no movement could be detected, and no change in 10 days. Then, fearing that the contents might be dead and disintegrating, I opened the egg case, and in it found eight little embryos with ciliated cells which gave them a slow rotation. In more developed specimens about half a dozen planarian embryos could be seen crawling over one another inside the cocoon; they were white in colour, each with two dark eyes. Hatching has occurred a few days after collection by way of an irregular hole at the distal end of the egg case, as many as eight little worms about half a millimetre long emerging. These were still quite white, and swam rapidly by means of their cilia or crept actively about the dish, while keeping constantly in the spot of brightest light. They were offered diatoms, etc., in the cultures, *Polystomella*, small copepods, alive and dead, pieces of a freshly



Text-fig. 4. *a*, *Polystomella* (the shell only being represented) bearing a turbellarian egg-case; *b*, emptied egg-case of turbellarian. *E*, egg case with developing embryos; *foss.*, fossette (d'Orbigny); *k.*, keel of shell; *ret.pr.*, ridges between the fossettes and over retral processes of the chambers; *ter.ap.*, terminal apertures of shell; *umb.*, umbo.

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[1] Also on a miliolinid (*Quinqueloculina*) from 'White Patch' in June 1941. It is not suggested that they necessarily have any special relation to the Foraminifera, although they are often so firmly fastened to the organic basis of the shell that they survive decalcification by acid. When they are found adrift from their substratum they proceed with their development and hatch as usual.

killed small gasteropod, and segmenting eggs of a mollusc: but were not observed to feed on anything of a size visible with a magnification of about 40. They disappeared about a week after hatching.

There is no operculum on the egg case, and it is interesting to note in one emptied case which was mounted on a slide that the chitinous capsule seemed somewhat macerated in the vicinity of the hole, i.e. its usually obscure tessellated pattern had become very obvious, as if a process of solution had been involved in the liberation of the young turbellaria.

It is probable that the 'gestielte, ansehnlich grosse häutige Beutel mit gerissenen Öffnungen am Rucken ihrer Schale festgeheftet' found by Ehrenberg (1839, pp. 109, 133, and pl. ii, fig. 1*g*) on *Geoponus stella-borealis* (= *Polystomella striato-punctata*) from Christiania in Norway, and on *Nonionina germanica* from Cuxhaven were also turbellarian cocoons—although I never saw one with the opening near the stalk; nor one with two or three openings such as he describes (1839, p. 168). M. Schultze (1854, p. 28), on the other hand, suggests that these were tests of the ciliate *Cothurnia*, such as he saw on the same species of *Geoponus* from Cuxhaven in the spring of 1851.

These cocoons cannot at present be ascribed to any known species of turbellarian. Of those already known they look most like minute representatives of the triclad bdellourid cocoons which so far have only been found infesting the gill-books of *Limulus*, the king crab, in both its eastern and its western areas of distribution (see, for example, Wilhelmi, 1909, p. 120). The egg cases on *Polystomella* are not flattened as are the bdellourid cocoons to fit into their special habitat between the leaves of the *Limulus* gill-books.

This is the only animal in any sense parasitic on a living *Polystomella* in my material. I have seen small algae growing on a shell during life, but the *Polystomella* already seemed inactive and soon signs of life ceased altogether.

Feeding

Observations were made in Petri-dish cultures with the aid of a Greenough binocular, and on the sides of taller vessels with a hand lens or a horizontal microscope. Finer details were seen by using a modification of an old method for continuously irrigating a culture over a microscope slide.[1] *Navicula mutica* was grown on cover-slips lying in a Petri dish, and when sufficiently covered, but not too thickly overgrown, the cover-slips were ready for use. A hole was punched[2] in a strip of filter paper (or two or three slips superimposed), and this was laid on a slide and moistened with sterile sea water. A clean *Polystomella*, preferably young on account of its greater transparency and activity, was placed on the slide in the middle of the hole and covered with sea water, then with a cover-slip with the diatom culture growing on its lower surface—the upper side having been previously dried off. The irrigation is very simply effected by strands of white (or 'natural') wool. I found it could easily be kept going for several days at least on the microscope stage when a single strand brought the sea water from a higher level on to the slide, and two strands led it away from the opposite side of the cover-slip into a lower vessel (see Text-fig. 5).

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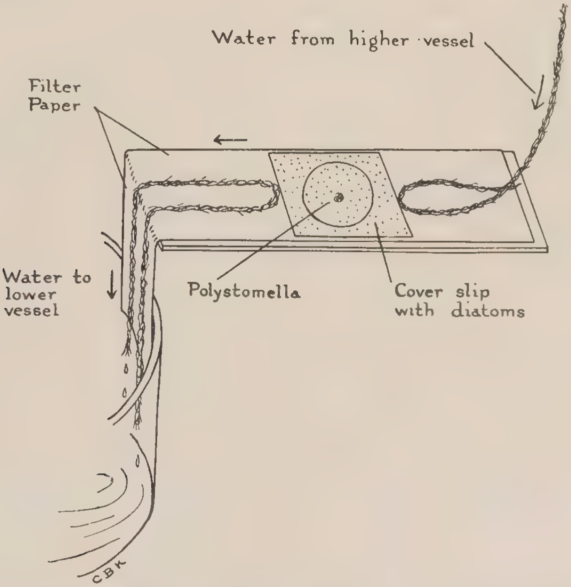
[1] See Schaudinn (1895*b*, p. 10). Also described by Lister in Vol. II of his laboratory notebooks (1892–1905).

[2] A sharp corkborer was used for this.

All that is necessary is to keep the upper vessel filled, and to wash the wool about twice a day in fresh water, rinsing it in sea water before it is replaced in position. In such an arrangement I have had a young *Polystomella* feeding for a week, in the course of which two new chambers were added, while protoplasmic activity inside and outside the shell could be observed under high magnifications. This activity was seen to be speeded up when the circulation was set going.



Text-fig. 5. Irrigation of living *Polystomella* on a microscope slide.

In spite of its massive shell *Polystomella* may be fairly mobile—quite often moving along as much as 1 mm. in 10–15 min.[1] by means of its very vivacious pseudopodia, which at the time are engaged in a great deal of other activity. This restlessness is not on the whole an advantage in working with cultures. I thought at times that the *Polystomella* tended to move towards a moderate light; but such a tendency, if it really exists, is not strong enough to be useful in controlling their movements under ordinary conditions. Dujardin (1835) also found the direction of incident light to be without influence on the positions taken up by his Foraminifera; but Verworn (1889, p. 40) claims that *P. crispa* moves slowly towards the source of diffuse daylight. I could not discover that their paths bore any relation to a current, for example, during irrigation. They do not appear to seek out good feeding grounds, except by random exploration; nor to stay on them when found, excepting temporarily while actually feeding. They creep up the vertical sides of culture vessels and may spend days crawling under the surface film where their food may be scarce, and whence they are not too easily dislodged once they have established a good set of pseudopodia there.

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[1] This figure agrees well with the speed of 4–8 mm. per hour recorded by Dujardin for his '*Vorticialis*' = *Polystomella* sp. (1841, p. 258).

The pseudopodia of various Foraminifera, including those of *Polystomella*, have been described several times already, and from different points of view (see e.g. Dujardin, 1841; Schultze, 1854, p. 16; Verworn, 1889; Schaudinn, 1893, 1895 *b*; Bütschli, 1894; Lister, 1903, p. 48; Winter, 1907, p. 49; Rhumbler, 1909, p. 251; de Saedeleer, 1932; Sandon, 1934; W. J. Schmidt, 1937; le Calvez, 1938).

Polystomella has pseudopodia of the *reticulose* type, like most of the group; that is to say they are narrow, almost thread-like, branched and continually anastomosing, usually by way of side streams, although occasionally two pseudopodia have been seen to flow together. Even in the finest end-twigs there is evidence of an active circulation, to and fro and across, in the lively movements of the many small granules which normally course up and down the pseudopodia of a well-fed healthy specimen. Sometimes a single granule will suddenly reverse its direction and jostle a way back a certain distance amongst the others which are in the meantime keeping on its former path.[1] This takes place even close behind the steadily advancing hyaline tip of an extending pseudopodium, or in one which is slowly contracting in the act of drawing food towards the shell; and the coloured excretory granules (to be described later), being caught up in the same movement, go to and fro on their way to rejection by the pseudopodia. The pseudopodia of *Polystomella* arise for the most part from the thin covering of cytoplasm which passes through the minute pores found all over the shell between the tubercles (Text-fig. 9*b*) and comes to lie outside it—but also directly from the protoplasm inside the shell via the terminal apertures, sometimes stretching right across the last chamber which is often not filled by the animal; and from the external openings of the canal system in the fossettes and on the umbo (Pl. IV and Text-fig. 4*a*). They may be shot out a short distance into the water like little rockets, and with the granules chasing up and down,[2] wave about like minute feelers, bending, undulating, quivering, and putting out side branches which meet and fuse and so establish the reticulum. This spreads, often in the form of a cone based on the shell, until in half an hour or so bundles of pseudopodia may lie more or less all round the shell reaching out for rather more than its diameter on every side, or for two or three times as far in one direction as the case may be (see Pls. IV, V).

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[1] The animation of the pseudopodia could best be shown in a film, and it was hoped to make one in collaboration with Mr A. G. Lowndes, formerly of Marlborough College, Wilts, whose ultra-rapid cinema-photomicrography (1935) has given such good results with small flagellates. A beginning was made, but the project has had to be abandoned for the present. See Pl. V 9, 10, 11.

[2] Rhumbler (1909, p. 255) ascribes a spontaneous movement to the individual granules in addition to that due to the cytoplasmic streaming.

The pseudopodia seem to be bathed in mucus, which is left behind as a trail after their withdrawal. Here and there in the pseudopodia are spindle-shaped varicosities, hyaline, not very refractile, varying in size up to several μ in length. They are deformable, divisible, etc., and moved along in the general streaming of the protoplasm. Sometimes a small one may remain close to the tip of a pseudopodium forming a kind of 'end-knob' (cf. Winter, 1907, p. 50) which may be sticky (Text-fig. 6*d*). Sometimes they spread out to make small webs where the pseudopodia branch, or flattened knots where several lie near together (Text-fig. 6*a*). They become very abundant in the pseudopodia at certain times—at a particular stage of the life cycle or after injury—and may be of a mucous nature.

The circulation goes on in the pseudopodia as long as they are extended, and in a well-developed reticulum is a very wonderful sight. Normally the pseudopodia are withdrawn by a reversal of the process of extension, but when necessary at lightning speed,[1] leaving behind droplets of various sizes (? of mucus) in their tracks.

I could not make out a definite axial thread in the pseudopodia of *Polystomella*, such as is present in those of the Heliozoa for example, although it seems probable that a more fluid surface layer, in which the granules principally circulate, gradually passes over into a core of more solid protoplasm. The pseudopodia show a fairly high degree of stiffness; they extend in a straight line as a rule, and may stretch unsupported through the water for a distance at least two or three times as great as the shell diameter.

Polystomella depends for its nourishment more than usually on its pseudopodia, for not only does it come in contact with food and catch it by means of them, but the process of digestion also takes place here. No solid food is as a matter of fact ever found inside the shell. Williamson (1849, p. 166) refers to 'very minute siliceous organisms—placed in positions indicating the possibility of their occupying the interior of the segments' in his dried specimens; these were probably the same as his '*Cocconeis*' of 1852 (p. 126), 'frustules of minute Diatomaceae' which he had 'recently found' in the interior protoplasm of a *Polystomella*—none too large to have passed through the terminal apertures of the shell—and which he evidently regarded as food. They are almost certainly loose foraminal plugs (see Text-figs. 6, 7). Lister, in his laboratory notebooks (1892–1905), several times mentions 'algae', 'parasitic algae', especially in the terminal chambers, 'with thick walls' or 'collapsed', and his sketch in Vol. 1 (opposite p. 111) puts it beyond doubt that these were in reality foraminal plugs, cast off probably at fixation (see p. 627). In 1895 (pl. vi, fig. 10) he figures the same structures in situ, without comment. Le Calvez (1938, p. 236)

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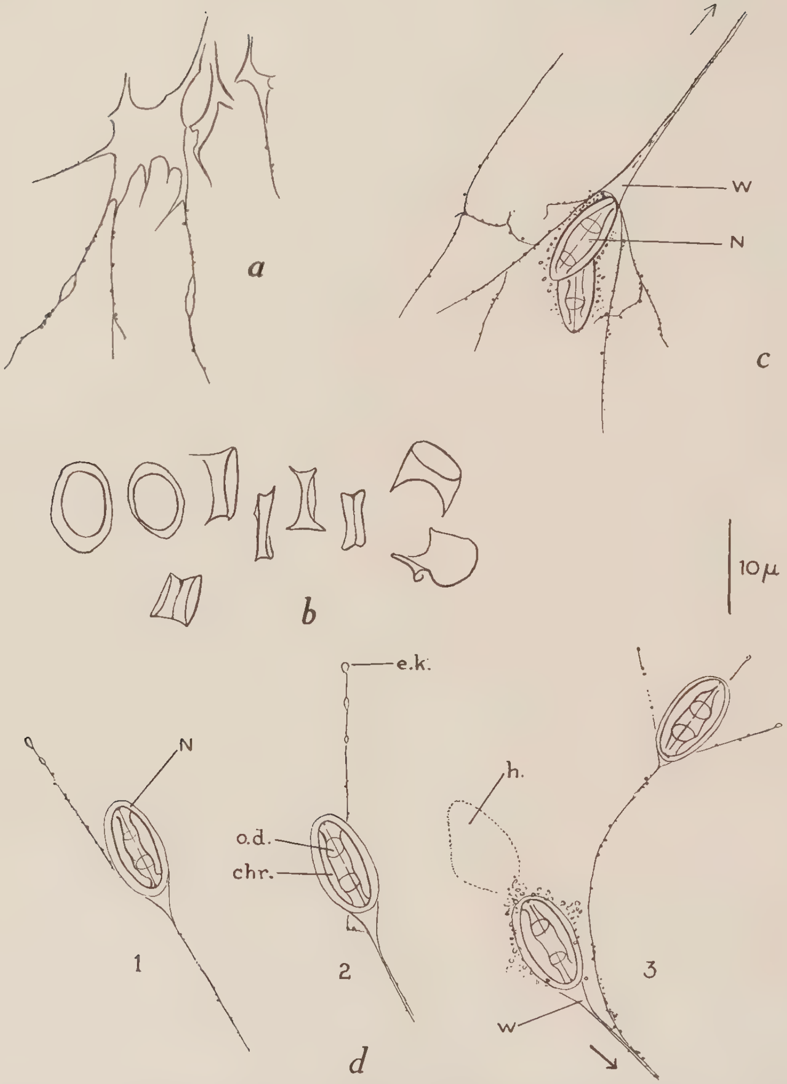
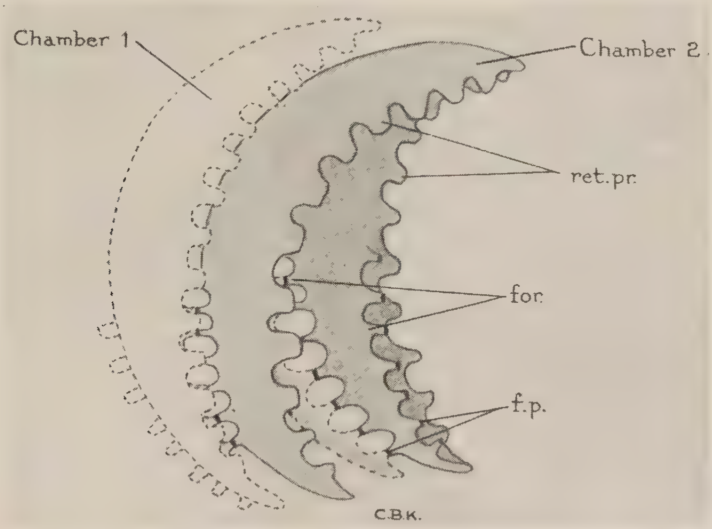
[1] These movements should be regarded as a resultant of cytoplasmic streaming and true stretching and contraction, as suggested by Schmidt (1937, p. 590).



Text-fig. 6. *a*, pseudopodia of an injured *Polystomella* showing a great development of ? mucous varicosities and webs; *b*, foraminal plugs sketched after ejection; *c*, pseudopodia showing a bundle of diatoms being drawn towards the shell in the direction of the arrow; *D*, 1, 2, 3, sketches at intervals showing the extraction of a small portion of the diatom culture on which the *Polystomella* was feeding, as described in the text, p. 628. *chr.*, chromatophore of diatom; *e.k.*, sticky end-knob; *h.*, hole in pasture; *N.*, *Navicula mutica*; *o.d.*, oil drop; *W.*, web attached to food mass.

first attempted to describe them fully, in *Planorbulina mediterranensis*. According to le Calvez the foramen is gradually closed up by a deposit which appears as a ring at the narrowest point and spreads across the opening to form a 'bouchon'. Periodically the plugs come loose and are carried out of the animal by the cytoplasmic currents. The cycle is synchronous throughout all the chambers. My observations on *Polystomella* indicate that the same thing happens here also, the plugs being composed of some 'chitinous' material, staining as le Calvez says with chromatin stains, including Feulgen, and showing in favourable specimens at all the foramina from the initial chamber outwards in both microspheric and megalospheric phases. Le Calvez does not give details of his observations on the shedding of the 'bouchons'. As already noted they are loosened by fixatives, especially when used hot, but they are certainly also cast at times in the absence of such unnatural treatment, for they appear along with the excretory granules in the dejecta of the living animal (see Text-fig. 6*b*). Their function seems at present quite obscure. (William-



Text-fig. 7. Showing the protoplasmic structures filling two chambers of the shell. *Chamber* 1 (outline dotted), *chamber* 2 (shaded), protoplasm in chambers; *for.*, protoplasm in foramina before and behind chamber, forming bridges by way of which each chamber communicates with its neighbours except in so far as the bridges are closed by the foraminal plugs *f.p.*, in situ; *ret.pr.*, protoplasmic retral processes underlying transverse ridges on exterior of shell. The canal system lies in the shell substance between these structures; a spiral canal, leaving the initial chamber and following the ends of the series of chambers, runs in the umbo on either side, giving off (*a*) in the septa, meridional canals which pass out between the retral processes and the foramina and have branches opening into the fossettes, and (*b*) several vertical canals which open in depressions on the umbo. After Williamson (1849), and see Carpenter (1860).

son's reference in 1849 (p. 165) to a constriction seen at times in the foramina of *Polystomella* must indicate a stage in the laying down of these plugs.)

When the pseudopodia of a *Polystomella* in the course of its wanderings come across a movable solid body they may get hold of it, and by subsequent appropriate shortening gradually draw it up towards the shell, perhaps pulling it in a millimetre or so in 10–20 min. Sometimes the object may go backwards and forwards on the way as the pseudopodium stretches and contracts again, and sometimes it is abandoned altogether. A food body may be seized in either of two ways. I have seen one come against the side of a pseudopodium which suddenly bends over it—thus enclosing it—or the tip of the pseudopodium may attach itself to the food body—perhaps by the aid of a sticky 'end-knob'—spreading out against it in a fanwise manner and apparently flowing more or less around it. Then it seems as if the food is actually pulled along towards the shell, while new pseudopodia may arise beyond it and go further afield (see Text-fig. 6*c*). It was most interesting to watch pseudopodia pulling on *Navicula* growing in a carpetlike mass of bacteria, etc., until a little patch would suddenly give way and be dragged out of the carpet, leaving a hole where it had been, as shown in Text-fig. 6 *d*, 1–3, while the patch was drawn away towards the shell. When the pseudopodia are working fast the collection of food (? embedded in mucus) may be enough to form a good 'feeding cyst' completely covering the shell in an hour or two. Inside this, the pseudopodia being withdrawn so far, digestion of the meal goes on. After an interval pseudopodia again appear outside the feeding cyst and the *Polystomella* begins to emerge. Sometimes a new chamber is first formed inside the cyst; and the following laboratory notes give an account of an emergence which I watched, from a cyst which happened to be thin, mainly composed of small granules with only a few diatoms in it. As is usually the case it was somewhat thicker over the terminal face of the last chamber, and this part is referred to as the 'face-mask'. '8/8/39, 9.45 p.m. R. had moved a very little way back from 'face-mask', but still in slough. Large numbers of rather straight parallel pseudopodia over face and parts of cyst nearby—i.e. above and below 'face-mask'. Lengthened as if pushing, while great numbers of pseudopodia on opposite side [of shell] pulled, and *Polystomella* slid backwards out of thin granular slough—pseudopodia passed easily through this.'

There had probably been but little feeding in this particular cyst, but this does not affect the mode of emergence, which may, however, be above or below the 'face-mask' instead of directly opposite it. Emergence from a complete covering of food is usually at the periphery—probably a line of weakness in the feeding cyst, and the point at which the strong keel of the shell could be most useful in forcing a way through. There is another record in July 1939 of a *Polystomella* which had also made a new chamber inside its cyst, this time mainly composed of *Navicula mutica*, and then had left it backwards as usual. The cast slough was examined. The majority of the *Navicula* in the cyst looked normally healthy, but amongst them were large numbers of empty frustules, the two valves of many of them being separated. There were also coloured excretory granules, about 2μ in diameter, especially in the 'face-mask'. This is the usual constitution of a discarded feeding cyst of *Polystomella*; inside this one the creature had evidently taken a meal, excreted, and made a new chamber. In Pl. V, figs. 4–6, are reproduced sketches made during life of another *Polystomella* which was watched as it fed on the planktonic diatom *Nitzschia*, while wandering on the side of an upright museum jar. First there was an abandoned feeding cyst (Fig. 4) composed, as could be judged from its colour, largely of diatoms; then another slough of a brighter golden hue was left behind (Fig. 5), largely made up of excretory granules. The *Polystomella* itself is seen moving off (Fig. 6). It seems that feeding may take place at any time of day or night. The interval between one meal and the next may be very short—a *Polystomella* is on record as making and discarding two feeding cysts in 7 hr.—or it may be a matter of days, according to circumstances. The actual digestion of the contents of a *Navicula* for example would seem not to take a long time, as so few are ever found in a condition intermediate between normality and the empty open frustules. These were observed mostly over the flat surfaces of the shell in one case—the undigested diatoms tending to lie near the periphery of the cast cyst. All my attempts to witness the process of digestion in detail have so far been in vain; and from what has been said it will be plain that success in this must depend on a very lucky chance, as it evidently takes place close to the outside of the shell, either in pseudopodia or in the external protoplasmic layer which is at times thick enough to enclose diatoms for example. It is interesting to note that the cast cysts of *Polystomella* feeding in *Synedra* cultures also show emptied frustules, although this genus has no open raphe. The valves may however be pierced by pores.

The 'coloured excretory granules' already mentioned are of special interest because they are such a conspicuous feature of the Foraminifera as a group. They are known as 'xanthosomes' on account of their amber colour (see Jepps, 1926; le Calvez, 1938, p. 268). They may be seen in trails (tracks of pseudopodia) and clumps in diatom cultures, etc., in which *Polystomella* have been feeding, as well as in their discarded feeding cysts. They also appear, at times in very great numbers, scattered through the cytoplasm of the living animal, or collected in certain parts of it. In *Polystomella* they vary in size up to 5μ or so across, and are often slightly angular in contour. Reference has already been made to their presence in the pseudopodia (p. 624), where they go about in the circulation for a time and are ultimately left behind by the protoplasm retreating from the mucous trail. We have seen also that they are sometimes thrown off in a more or less complete cyst-like covering. Like the foraminal plugs which are sometimes thrown out with them, they are too large to pass through the fine pores of the shell. They may be seen massed in the 'face-mask' about the terminal apertures (foramina) of the last chamber, in the fossettes between the retral processes, or in the depressions on the umbo of the shell. Both xanthosomes and foraminal plugs are present in the protoplasm of the canal system in a number of my mounted specimens. The former can be shown in the terminal apertures as well as in the position of the fossettes; while the latter tend to collect in the latest formed chambers and perhaps usually pass out by the foramina, although in at least one preparation appearances suggest that they also may be able to leave the shell by openings into the fossettes. Excretory granules have actually been seen on their way out in strong pseudopodia emerging from the fossettes of the last two chambers and from the terminal foramina of a *Polystomella* under irrigation. Thin discarded feeding cysts often show a pattern representing the fossettes of the lower side of the shell in little heaps of excretory granules. The conclusion is that these solid bodies pass out of the shell by one or other of these two routes —and therefore that the canal system of *P. crispa* certainly has openings into the fossettes as described for the larger *P. craticulata* (up to about 5 mm. in diameter, fossilized), from the coast of Australia by W. B. Carpenter (1860), although he specifically denied it for *P. crispa*. It is easy to understand how Carpenter came to pass them over. I could not be quite certain in my preparations of more or less normal whole *Polystomella* that the meridional canals (see Text-fig. 7 legend) bore lateral branches which might open into the fossettes until I had seen them most clearly displayed in one of a set which had been living in a culture of *Synedra* for some months. Owing possibly to more than one cause, the protoplasm was badly diminished and disorganized—the nucleus had disappeared—but the canal system seemed swollen out and full of staining granular material so that it was very conspicuous indeed, even to these ultimate branches, one to each fossette, especially where it had broken away from the remains of the chambers. The canals are of course to be seen fragmentarily in sections of *Polystomella*. My observations on the canal system confirm the findings of Lister (see 1903, pp. 65, 66, with fig. 9).

It is often stated that the pseudopodia of Foraminifera have the power of killing their living prey suddenly as if by the action of some specific poison (Schultze, 1854, p. 23, for *Polystomella* and *Gromia* feeding on 'kleine Infusorien'; Winter, 1907, p. 10, for *Peneroplis* feeding on small crustacea; and others). I have seen no evidence of this in *Polystomella*; but I have not yet offered small crustacea as food, and Winter says the poison of *Peneroplis* is not so effective against 'Infusorien' and 'Flagellaten'. As already indicated my *Polystomella* fed mainly on diatoms, though there were usually plenty of small zoomastigine flagellates ('*Bodo* sp.') about. Occasionally, however, I have seen these being drawn towards the shell. They continue their movements for some time, even after they begin to look rather abnormal, and seem to die gradually in the course of their transport. This agrees with the observations of Rhumbler (1909, p. 253), although I cannot agree that the movement of the prey is necessary to its capture as he goes on to suggest (quoting Verworn, 1889, pp. 148–9). We have seen that non-motile diatoms, for example, are caught, and apparently used as food. Besides these, *Polystomella* frequently takes inanimate objects up to its shell, such as small sand grains, filter-paper fibres in the irrigation experiments, etc., and manufactures a more or less complete covering out of them. Although I have a general impression that *Polystomella* did to a certain extent choose diatoms, and particularly *Navicula mutica* as food in the cultures, it is difficult to say anything more definite about its power of selection in the face of the probability that the function of the cyst may be partly protective (see p. 642). Further, although Cryptomonads and Chlamydomonads when available were also incorporated in the cysts, in spite of a search no evidence was obtained that they had been used as food; but even if they had been, neither would leave as conspicuous an indigestible residue behind as the frustules of a diatom. Some other Foraminifera, including the pelagic forms, are reputed to feed to a large extent on animal food, especially small copepods (see for example Rhumbler, 1900, p. 2; Winter, 1907, p. 10), and it is hoped, when opportunity offers, to try the effect of a more mixed diet on *Polystomella*.

Foraminifera have long been famous for their tenacity of life, at least in certain circumstances (Schultze, 1854, pp. 21, 31). Although at times their appetites seem large, some of them, including *Polystomella*, can survive long periods of more or less complete starvation. When short of food *Polystomella* slowly loses its brown colour, at any stage of its life history, often becoming patchy and then always paler until in 3 or 4 weeks it may be almost white. If it has not degenerated too far, the colour will return when food is again taken, a week or 10 days of good living making a noticeable difference. As pointed out by Rhumbler (1909, p. 255 footnote) the granules gradually disappear from the pseudopodia of a starved *Polystomella*; all the cytoplasm in fact seems to become thinner in its consistency, and inside the shell it is seen to decrease in extent. Lister (1895, p. 422) gives figures to show that starvation also induces a shrinkage of the nucleus relative to the cytoplasm as indicated by the number of chambers filled at the time of fixation. It is a pity that his two groups of *Polystomella* were collected at different seasons, viz. 118 fixed fresh from the sea in May and June, and forty-eight collected in October and fixed in January after 3 months in the laboratory on short rations; because, owing to the annual cycle of which Lister was unaware, there is also an age factor involved, though it is not known that this actually affects the result. The curves come out remarkably clearly for so comparatively few specimens, and in spite of the fact that the number of chambers is only a very rough indication of the *amount* of protoplasm present since they vary a good deal in size.

PART II. THE BIENNIAL CYCLE, INCLUDING AN ACCOUNT OF THE LIFE OF A MEGALOSPHERIC BROOD IN CULTURE AND A DESCRIPTION OF THE FORMATION OF A NEW CHAMBER

The Microspheric Phase

It is well known that microspheric *Polystomella* often undergo asexual reproduction in a laboratory culture if provided with a modicum of food.[1] Schaudinn 'mentioned' the event (1894*b*, p. 42) and referred to it again with a few particulars (1895*a*, p. 59; 1903, p. 499). In 1895 Lister gave in his Postscript no. 2 (p. 445) an incomparably succinct account of the process after observing 'some hundreds of cases'. This was amplified in 1903 and illustrated with the now familiar figures (pp. 67–9). Later references (Lister, 1906, p. 7; 1907, p. 492) only added that some 200 young are commonly produced in a brood. Heron-Allen published extracts from Lister's Notebooks (1892–1905) giving further details of the actual observations, the most important being that 'in specimens whose protoplasm has begun to emerge, faintly stained round nuclei 10μ in diameter are found in the clear protoplasm of the terminal chamber' (1930, p. 7). This supports Lister's statements that there are nuclei in the young megalospheric individuals from the beginning, although, curiously, he had originally come to the same conclusion as Schaudinn that the nuclei of the microspheric parent had completely disintegrated into a chromidial form before reproduction set in. I believe that this conclusion may be due, partly at any rate, to imperfect fixation, because of a very severe reaction to disturbance, perhaps accompanied by actual injury to the protoplasmic mass in the course of securing it for fixation at a particular moment. Emergent protoplasm which was detached from the walls of a culture dish either showed no nuclei at all or nuclei somewhat altered in appearance, so as to make them even more easily concealed amongst the deeply staining chromidial bodies which are very conspicuous at this time. On the grounds of analogy and general theory one can accept complete nuclear dissolution only with the greatest reserve until it is demonstrated to be true beyond all question. There is no need for further discussion of the inaccuracies of Schaudinn's account of the process since they are fully dealt with by Lister (1903, pp. 69, 70).

Perhaps the sensitivity referred to above may account at least in part for the fact that we have as yet no description of any nuclear division in *Polystomella*. It is of course notoriously rare to come on most species of protozoa with nuclei in a state of division; and apart from the sporulation phase there are anyway only a few nuclear divisions in the long life history of a *Polystomella*. The structure of the nucleus was described by F. E. Schulze (1877, p. 17, pl. ii), and its position about midway in the series of chambers of the megalospheric phase of *P. striatopunctata* indicated. The nuclei of the microspheric phase are similar in appearance and are spread through the chambers forming approximately the middle third of the series, those in the outer chambers (as pointed out by Lister) being larger as a rule than the others. It may be said on the whole that the nucleus of a large megalospheric *Polystomella* is more likely to be packed full of nucleoli of a smaller size, which, however, may vary a good deal inside one nucleus, while, as noticed by Lister (1895, p. 419), those of the microspheric form tend to fewer nucleoli of a larger size, especially the larger ones in the outer nucleated chambers. No positive reaction to Feulgen's stain was obtained in the nucleus of either form. Both Lister and Schaudinn believed them to increase in number by direct (amitotic) division as the chambers increased. As 'good evidence' for this Lister indicates their disposition in the chambers in pairs of similar size and appearance, sometimes united by a narrow bridge as if in the act of dividing with absolutely no visible rearrangement of their components (1895, p. 419). These observations are easily confirmed, but I believe they may be found susceptible of a different explanation. It does not, however, seem profitable to discuss the matter further at present. After examining whole mounts of some 125 and sections of over 200 microspheric *Polystomella*, many of them in April and May when reproduction is at its height, I am still unable to give the smallest indication of any division figure. Some of these specimens were fixed soon after collection from the sea, i.e. in a few hours' time during which they were kept as far as possible at sea temperatures. Others were cleaned and put to feed in diatom cultures for periods up to a fortnight, and afterwards fixed at various times of day and night. Some were starved and then fed; a further trial of this procedure is greatly to be desired.

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[1] And occasionally if no food is provided.

Nuclear division in microspheric *Polystomella* seems to go on at any time of year if we may depend on size differences, etc., of the nuclei as indications of recent multiplication. Such indications are most often seen towards the middle of the series in each *Polystomella*, where the nuclei tend to be more numerous and smaller; and are evident in specimens collected in October and November as well as in April when asexual multiplication is generally imminent. If averages obtained from my comparatively small numbers can be trusted, there is not much increase in the number of chambers through the winter months, but the nuclei have multiplied from say thirty-three in the autumn to forty-four (varying ± 10) in some fifty-four chambers by the middle of April (thirty-seven specimens, 11 April 1940). About two further divisions for each nucleus are then all that are necessary to produce the number required by the brood of megalospheric young. The highest number of nuclei shown in my preparations is about 116 in a *Polystomella* of fifty-five chambers collected in March, the protoplasmic body after decalcification measuring a little over 1·5 mm. in diameter,[1] and having as many as a dozen or more nuclei in some of the chambers towards the middle of the nucleated series. Another *Polystomella* of the same size (in August) had seventy-six chambers, the biggest number I ever found; and the largest specimen (in the April collection) with sixty-eight chambers measured 1·8 mm. after decalcification. Microspheric *Polystomella* tend to reach a large size on the average, although some megalospheric forms also attain the same dimensions. I have no record of asexual reproduction in a *Polystomella* that was not over 1 mm. across in the shell and probably comprised at least fifty chambers.

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[1] My measurements refer to what is usually the greatest diameter, along a line passing lengthwise through a chamber a little before the last of the series. I regret that many were made before I realized that the official plane of measurement runs across the shell parallel to the last chamber. In counting the chambers I have reckoned the 'globular swelling' referred to by Lister (1895, p. 418), which lies next to the initial chamber as the second markedly smaller chamber of the series, which it undoubtedly is. The third chamber is roughly as big as the initial chamber, and thereafter there is generally speaking a steady increase in size in the

It seems that Lister was working with a population of a smaller size or an earlier maturity—for he quotes (1895, p. 419) as one of his largest microspheric specimens a *Polystomella* of forty-seven chambers which measured 800μ across (? after decalcification); while a few figures in his Notebooks (1892–1905) give an average of thirty-six chambers for ten reproducing microspheric individuals.

The *Polystomella*, which usually show signs of starvation by the end of winter,[1] are able to feed again as soon as the diatoms begin to increase in the spring. Their colour improves and growth again takes place. By early in March 1939 it was noted that they had begun to look a little browner in the collections, and by the end of the month some of them had taken on a rich brown colour. From now on it was possible to make a partial separation of megalospheric and microspheric forms. Apart from the fact that one may often effect a slight concentration of the microspheric *Polystomella* by selecting those of the largest size, in April they could be collected by taking those of the richest colour and those which showed the greatest recent growth. This was apparent in the shape of the creatures, because their newly formed chambers were conspicuously large, raising the contour of the shell into a high bulge as they spread over the whorl below. This feature is actually shown in Lister's figures (1903, pp. 63, 67) without comment. Though it is good enough to be useful in choosing specimens for cultures, the test is by no means infallible even at this season. Young or small megalospheric *Polystomella* which feed and grow at a greater rate, naturally tend to exhibit what we may call the microspheric figure, and there are always some larger ones of both kinds which are also exceptions to the rule. The degree of success in diagnosis of living *Polystomella* which was attained by these criteria is indicated by one or two examples. On each of two

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series. It might be pointed out here that the initial chamber of the microspheric form differs from all other chambers of both forms when seen in decalcified specimens in that it is enclosed in a thickish capsule, like a cyst wall with a single pore which is the foramen. It would obviously be more significant to give measurements of this capsule where possible rather than of the more variable cytoplasm which may not fill it, although the capsule itself appears shrivelled in many preparations. As Lister (1895, p. 418) observed, the first three or four chambers are also different in that they are arranged in a spiral, which may be either right-handed or left-handed. Does this represent an ancestral form of the creature? (See Lister, 1903, pp. 135–7; Tan Sin Hok, 1935; Ovey, 1938).

[1] It will be very interesting to compare my account of the annual cycle, based on general observation and cultures, with that of Earl H. Myers derived from his statistical investigation of very large numbers of specimens, during the same year at Plymouth.

occasions in May about thirty 'microspheric' *Polystomella* were picked out, mostly by the shape, and there were some which could not be assigned to either form. Only about 10 % were correctly selected. Better results were obtained in April by choosing the darkest brown specimens, especially the largest ones, when a 60 or 70 % success was reached. It is plain that the method could not be applied to individual specimens. It is usually stated that there are only about 3 % of microspheric forms in the population, and this is true of the greater part of the year. In the month of March 1939 the percentage rose from this value to about 25 %, and in April it continued to rise to about 30 %. This was due to the fact that the megalospheric forms had been sporulating[1] from January on, and so gradually disappearing from the living population. At first only a few *Polystomella* were reproducing, the peak of sporulation occurring late in March or early in April. It was perhaps at their point of highest frequency that the shape of the microspheric forms was most distinctive; later in May the choice became confused by the presence of some megalospheric young, and perhaps by the darkening of the colour of some older ones after more prolonged feeding.

A few comparable figures are given in Table I for small collections of *Polystomella* from the 'White Patch' ground in 1940.[2]

TABLE I

Date	% microspheric form	Total collection
3. i. 40	10	250
29. ii. 40	17–18	90
5. iv. 40	about 50	94
18. vii. 40	about 7·0	about 300
21. x. 40	4·5	424

Some time in April the microspheric *Polystomella* in turn reached the peak of their season for reproduction by a form of schizogony, and in turn began to show a notable reduction of their numbers.[3] This asexual reproduction began early in February in my cultures fed on *Navicula mutica* for a little over a month, and in the sea by the middle of April there were already a large number of young megalospheric forms, 0·5 mm. or so in diameter, offspring of this process. On 26 April I selected twelve *Polystomella*, measuring 1·2–1·6 mm. in diameter, as likely to be microspheric from their colour and shape, and nine of them produced young asexually in cultures of *Navicula mutica* between 5 and 22 May (at least two of the other three were megalospheric). During this time there was unfortunately an unavoidable interval in my observations, and when I returned to them on 22 May the high season for schizogony seemed to be past, although it occurred in my cultures later on, and probably continued in the sea through the summer months.[1] From June onwards large *Polystomella* of any kind were scarce in the catches, and of these a fairly high proportion were microspheric. Of eighty specimens in August 6% were microspheric, and young of 10–20 chambers were still common in October. By the end of June some young had reached the size of the smaller specimens of the year before, i.e. a millimetre or so in diameter, but they could still often be distinguished by their browner colour. Towards the end of July a feeding experiment indicated that less food was being taken and that the rate of growth had very much slowed down, and in August I could no longer pick out 'young' and 'old' specimens amongst those of the larger sizes.

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[1] This process will be discussed in a later section of this series.

[2] No great value must be placed on the exact numbers given. The samples were not especially taken for this purpose, nor all in the same way.

[3] Heron-Allen (1914, p. 9, footnote) refers to the finding of 'a very large number' of two- and three-chambered megalospheric young on the Drake's Island ground in April 1929.

Of the *Polystomella* from 'White Patch' to which reference is made in Table I about 100 were put to feed in a culture of diatoms on 20 July 1940, and eight out of twelve microspheric individuals reproduced before 27 August, while a similar experiment in October gave no reproduction at all.

Development of the Megalospheric Phase

Some of the young megalospheric *Polystomella* produced in my cultures were reared for varying periods; and some account will now be given of the more interesting observations on two out of several families born in Petri-dish cultures of *Navicula mutica*. In both of these the parent shell was incompletely emptied—but in others all the protoplasm came out as it probably does in the wild, leaving a very clean white shell behind. Such shells often have holes in the wall of the terminal chamber, apparently made by the issuing protoplasm. The diatom cultures were of course renewed as required, and larger vessels used as the young grew up.

The largest Petri-dish brood (N 4) numbered some 150 young. On 3 and 4 February 1939 a *Polystomella* measuring a little over 1·5 mm., which had been feeding on *Navicula*[2] in sea water since 17 January on the laboratory bench at temperatures of 55–60° F., was seen under the surface film, supported by a large number of long pseudopodia. On 7 February it was still at the surface, with almost all its protoplasm outside and divided up into the family of two-chambered young, which were engaged in collecting diatoms and ? bacteria out of the surface film into their pseudopodia.

8 February. Many young have a third chamber.

9 February. Young spreading over the surface film. Some with four chambers.

10 February. Four to five chambers. Renewed the sea water carefully with a pipette.

11 February. Put a few young on the bottom of the dish where there was more food.

18 February. Parent has fallen to the bottom. The brown protoplasm remaining in the last few chambers is putting out pseudopodia. Wall of terminal chamber seen to be damaged. Some young with six chambers.

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[1] J. J. Lister studied this method of reproduction in *Polystomella* from the English Channel in the months of May, June, and July.

[2] Grown in Erdschreiber, which was replaced by sterile sea water when the Foraminifera were introduced.

23 February. $4\frac{1}{2}$ chambers of parent shell seen to be full of protoplasm. Young on bottom with eight to nine chambers. On surface where food is scarcer, six to seven chambers.

26 February. Young on bottom much better grown than at surface, where diatoms are very scarce.

28 February. Young on bottom with ten to eleven chambers.

3 March. Parent shell with five to six chambers full.

4 March. Young up to one dozen chambers. Some which started with irregular growth now seen to be growing regularly.

7 March. Biggest young with fifteen to sixteen chambers.

14 March. Cannot now count chambers of big young in shell. Parent shell with protoplasm in $8\frac{1}{2}$ chambers.

8 April. Young up to over 0·5 mm. Fixed some, about one dozen chambers. Diatoms not very good now. On 7 March parent and some of the best young were transferred to a fresh culture of *Navicula* in Erdschreiber. These young now reach 740 μ. Parent shell has protoplasm in almost all exposed chambers.

30 April. Most young about 1 mm. across, one is 1·2 mm. One small, with only seven to eight chambers ('dwarf'). Young look well, but parent very pale and starved looking. Diatoms going off.

7 May. Taken to cooler room—at 13° C.

28 May. Some young up to 1·3 mm., with about forty chambers. Parent probably died about now.

14 June. Dwarf shell empty—about 400 μ.

23 August. Fixed sixteen young, brown in colour, and one very pale with greatly reduced protoplasm, measuring 1·1–1·5 mm.; and two smaller pale ones, also degenerate and measuring under 1 mm.

Another brood (N 2) was born in a similar culture of *N. mutica*, but kept in Erdschreiber at 55–60° F., between 17 and 23 February. The parent shell measured about 1·6 mm. across and had been in the culture since 16 January. In the interval its colour had darkened to a very rich brown shade, especially near the terminal chamber, and it had probably grown one or two new chambers. It was found up the side of the dish on 23 February, a good deal of the protoplasm remaining in the shell and part of that which had emerged undivided. Some healthy looking young were close by—but only thirty-five were ever recovered in this culture. The diatoms were especially good.

7 March. Parent shell has one to two very irregular new chambers.

14 March. A few young have fifteen to sixteen chambers, one only two to three. Many are abnormal and there are some empty shells.

16 March. Parent shell now has about eighteen chambers full of very dark protoplasm.

7 April. Parent shell now has protoplasm in all visible chambers.

30 April. Average diameter of young shells about 800 μ. There were twenty-six good young and nine very deformed. Some fixed in Susa showed about twenty-six chambers, also that individuals which appeared to have begun life as monsters came to grow quite regularly after a time, even when two nuclei were present (one pearshaped initial chamber), presumably owing to imperfect division of the parent protoplasm at schizogony.

27 May. There are twenty-five young now, up to 1·1 mm. across.

28 May. Put in cooler room, at 13° C., but kept at various temperatures (say 13–17° C.), during the summer months.

23 August. Fixed the parent. The protoplasm was a very dark colour, but the shell was highly irregular near the terminal chambers. In section the whole series of chambers was found to contain protoplasm, with about a dozen nuclei and a few shreds of chromidium. It is interesting to observe that the solitary nuclei which do not appear to have undergone recent division are quite full of small nucleoli, looking like the nuclei usually characteristic of a megalospheric form.

2 September. Young now over 1 mm. in diameter, in many cases with some irregularity towards the end of the series of chambers. Rather pale.

23 September. Taken to Glasgow in cultures of *Navicula mutica* in jam pots, and kept there at about 15–16° C. The cultures became rather poor and the *Polystomella* paler.

8 November. Moved to better cultures and improved in colour.

21 November. The *Polystomella* in about 2 l. of Erdschreiber in a large glass basin grown with *Navicula mutica*, were placed in a sink with running cold water[1] at a temperature of 11·5° C. (sea temperatures recorded at Plymouth Pier in Nov. 1939 about 13·5° C.). *Polystomella* now quite a good colour, measure up to 1·3 mm.

15 December. Temperature down to 9–10° C. (sea temperature at Plymouth fell to 10° C. by the end of the year 1939). Careful inspection showed the *Polystomella* of a good colour, but no evidence of recent growth.

18 January 1940. *Polystomella* inspected almost daily since 8 January, still brown.

20 January. One *Polystomella* looks pale. Temperature in the sink this month about 8° C.

21 January. Temperature down to 6·0° C.—very cold weather (sea temperatures at Plymouth down to about 7·5° C. by the end of January 1939). Basin was broken, and had to be left so that the culture was at about 10° C.

9 February. Two *Polystomella* now look almost white, one with brown patches.[2]

14 February. Twenty-three brown *Polystomella*, and two almost white ones which look dead.

17 February. Two very pale *Polystomella* crushed, and the protoplasm seen to be divided up into spheres of 4 μ to two or three times as big, with shining inclusions. One more *Polystomella* looks pale, the rest seem to be feeding well.

23 February. Another *Polystomella* 'white', seen next day to have brown patches on its lower side, in the fossettes.

26 February. Pale *Polystomella* of the 17th is colouring up again. 'White' one of 23rd crushed and found to contain dead rounded up flagellispores with shining inclusions. The brown patches seen to be excretory granules. Temperature this month varying 1–2° on either side of 10° C. (sea at Plymouth in January 1939 about 8° C.).

9 March. Temperature 9° C. Four 'white' *Polystomella* seen: (1) First seen to be white on 7 March and almost certainly not so the previous day. About noon the shell was observed to be full of active flagellispores, with some excretory granules. Examined at intervals all day, but no emergence seen. Next morning the activity had ceased—the little flagellates apparently having died in the shell. (2) Crushed on 10 March, cytoplasm dead, unsegmented. (3) Crushed on 10 March, cytoplasm dead, mostly segmented. (4) Crushed on 10 March, cytoplasm dead, segmented into spheres and distorted flagellispores, both showing some activity. No more 'white' *Polystomella* were seen.

19 March. Left eighteen young in culture, three rather pale, and went to Plymouth.

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[1] We are fortunate in Glasgow in having a plentiful supply of water from the Trossachs which runs very cold all the year round, and shows a seasonal variation in temperature which happens to be only a little greater than the shallow water temperatures recorded from the pier at Plymouth.

[2] The significance of some details now given will appear in Pt III of this paper.

The temperature in the culture slowly rose from 9·5 to 11° C. by the time of my return (sea at Plymouth only up to about 10° C. at this date in 1939).

21 April. Eighteen *Polystomella*, no empty shells; two or three pale, but some very brown indeed in places.

30 April. Crushed three pale *Polystomella*; all dead or degenerate. The *Navicula* cultures became unsatisfactory and the *Polystomella* were transferred to cultures of other diatoms.

18 May. They were still feeding and mostly a good colour. Temperature about 13° C. (sea at Plymouth up to about 13·5° C. by the end of May 1939).

29 June. Fourteen *Polystomella* apparently still feeding, but pale or patchy in colour. Hot weather. Temperature up to 19° C. (sea at Plymouth in June 1939 about 16·5° C.).

12 July. All very pale. Left to feed in a good culture of mixed diatoms during vacation. Temperatures probably about 16–17° C. (sea temperatures recorded at Plymouth Pier July–August 1939 about 16–17° C.).

14 September. Culture now pale and patchy, though there are remains of a good growth. *Polystomella* found: six of a good colour, 1–1·5 mm.; nine very pale; two greenish, look dead. Some of the shells were preserved as dry mounts, five crushed. Contents of two pale and two greenish ones very unhealthy or dead.

These culture records show how rapidly the young megalospheric *Polystomella* may grow. Lister noted that two chambers may be formed on the first day (Notebooks, Vol. II, p. 116) and another two by the end of the second day (1903, p. 69). Actually an average rate of a new chamber almost every second day was maintained for about 3 months, by which time the young measured a millimetre or so across the shell and had some forty chambers. In June 1939 some young *Polystomella* from the sea, measuring about 400μ, formed another new chamber every other day with great regularity up to a period of 10 days in cultures of *Navicula mutica*. In July a similar experiment indicated that there was some falling off in the amount of food taken and in the rate of growth in *Polystomella* which already measured about 1 mm. Probably the average number of chambers finally attained is somewhere between forty-five and fifty; although one large megalospheric shell collected in January to keep in culture measured 1·8 mm. and was found to have contained sixty-seven chambers at the time of its collection.

No suggestion of a repeated schizogony was ever seen in my cultures.

The history of culture N 2 shows that at the end of a year some of the *Polystomella* attempted to sporulate. This seemed most surprising after so many vicissitudes in the course of their lives, and showed that it is not difficult[1] to keep these Foraminifera in cultures up to this critical stage, which as already noted will be the subject of further discussion in a later part of this series.

It is time now to refer to an unexpected imperfection in the results obtained in my cultures. In both the grown *Polystomella* taken from the sea in January 1939 and of the young reared from them at Plymouth it was found that the shells tended to become abnormally heavy, apparently owing to an extraordinary secondary deposit of shell substance which tends to fill up all the depressions, thickening the prominences and gradually obliterating the normal shell pattern. One such case is illustrated in Text-fig. 8, drawn from a sketch in a laboratory notebook in August 1939. This *Polystomella* had lived in *Navicula mutica* cultures since 17 January. It appeared as if the openings in the shell might be occluded, but several of the vertical canals opening on the umbo could still be seen and pseudopodia were put out while the sketch was being made. The same sort of disfigurement was seen in some of the young after a few months, and became worse the longer they lived in the cultures; the shells were thick from one side to the other, and did not usually reach as large a diameter as their parents, or as many wild megalospheric specimens. This general thickening is a different phenomenon from other shell abnormalities such as:

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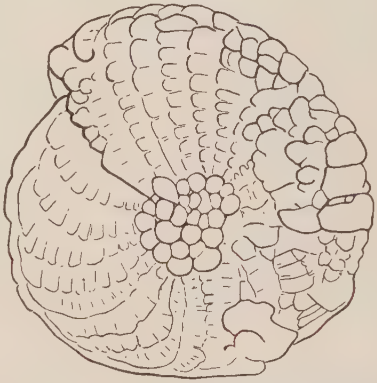
[1] They might have done even better if they had begun life in larger vessels, for instance, or if the temperature had been more consistently controlled. It is hoped to repeat the rearing of a megalospheric family under better conditions when supplies again become freely available.



Text-fig. 8. *Polystomella* with abnormally thickened shell, after some months in culture; cf. Text-fig. 4 *a*. Some of the young reared in cultures into their second year became much worse than this.

(1) The irregular growth which often occurs in very young megalospheric offspring of schizogony, especially perhaps in cultures but not exclusively there, and which as we have seen may right itself after the formation of a few irregular chambers.

(2) The real monsters which develop when, e.g. two young grow as a twin, two initial chambers being formed.[1] Possibly such a beginning may lead to grown-up monstrous shells like those depicted by Schultze (1854, p. 30, pl. v), with more than one whorl of chambers. Other abnormal shells may also occur in the sea, e.g. with the chambers taking on a rectilinear arrangement at a certain stage (see Millett, 1904, p. 604, pl. xi) and cf. *Ozawaia* Cushman (Cushman, 1933).

(3) Shells with small irregular new chambers, such as are formed after injury to the more or less naked protoplasm at an early stage of the process of their formation.

The thickening is obviously due to some cultural condition, and at present it is not possible to account for it. Heron-Allen (1914, p. 262) relates how he got extra ridges, etc., on *Massilina* shells in cultures when he replaced the water of evaporation with hard water from his well. But thanks to Dr J. D. Robertson, of this Department, who very kindly made the necessary estimations, I can say that my culture media only contained about the average amount of calcium in sea water. Unused medium (Bearsden earth extract + Clyde sea water) contained 409 mg./l.; while after nearly 4 months use by a culture of diatoms and *Polystomella*, another sample (Plymouth earth extract + Clyde sea water) contained 384 mg./l.; the salinity of the two samples being within 1 % of each other, indicated that there had been no appreciable evaporation in the used medium.

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[1] Quite regular adult megalospheric specimens with more than a single nucleus occur in the sea (see also Lister, 1895, p. 425). But these have only a single initial chamber, so that if they indicate twinning it is here more complete from the outset (see also Heron-Allen, 1914, p. 249).

Another peculiarity of the several hundreds of young I saw in my cultures was that not one ever showed the least sign of spines on the keel at the periphery of the shell, such as are shown in three- to four-chambered young by Lister (1903, p. 69) and are commonly seen on shells of small size (under 1 mm.) from the sea usually said to be young *Polystomella* of various species (see Williamson, 1849, p. 163, on young *P. crispa* 'when obtained from deep water'; Schultze, 1854, Taf. v, on young *P. strigilata*; Brady, 1884, on young *P.? macella*, and on *P. imperatrix* at all ages). Such small spiny shells are not infrequently present in the Drake's Island collections (down to about 5 fathoms) and are very common amongst the small *Polystomella* found in shallow *Corallina* pools on the shores of the Firth of Clyde (at Millport).

Regarding the actual emergence of the protoplasm and its subsequent fission, my observations, as far as they go, confirm the account given by Lister. As he reports the 'premonitory halo' of numerous colourless pseudopodia (1903, fig. 10, p. 67) is as a rule developed early in the day, between 6 and 10 a.m., the *Polystomella* often having crawled up the side of the culture vessel beforehand. Just as when a portion of the protoplasm emerges to form a new chamber, so here the pseudopodia are soon followed by the brown protoplasmic body, which appears to leave the shell by way of the canal openings near the last chamber[1] and probably by the terminal openings also. In some cases, as has already been mentioned, additional large openings are made in the wall of the terminal chamber as described in *Discorbina* (see Schlumberger, 1896) and *Peneroplis* (Winter, 1907, p. 23). As already noted Lister found small nuclei in his reproducing microspheric *Polystomella*; these went down to about 7μ in diameter (1903, p. 70). In my preparations there are none so small, all the nuclei being well over 10μ. In a few young of two to three chambers they measured 15–20μ, and at a dozen chambers about 30μ. They show nucleoli from the beginning; and indeed, as compared with a collection of young from the sea, a family likeness may be observed in the size, etc., of the nucleoli in a brood of young, as well as in the appearance of the chromidial mass, which may be very compact or more or less diffuse, scanty or plentiful. This is of course due to the protoplasmic structures being taken over from the parent. I am not sure whether the size of the initial chamber is more uniform within a family or not—there is evidence in my preparations of the measurements being affected by pressure of the cover-slip, and special observations would be necessary to determine this point. Retral processes appear on the second chamber of the young (see Text-fig. 1, F, A); the nucleus leaves the initial chamber when there are about a dozen chambers and wanders out to take up its position usually a little behind the middle chamber of the series. The chromidial masses have entirely disappeared soon after this stage, and the nucleus now has the numerous nucleoli usually present in the megalospheric phase.

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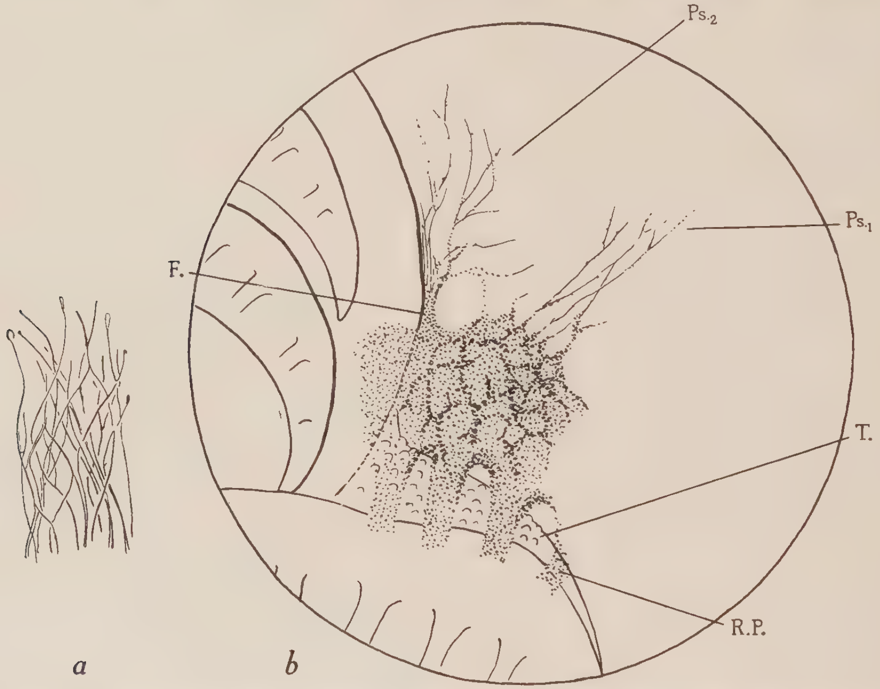
[1] In his Laboratory Notebooks (Vol. II, p. 131) Lister says that during emergence of the protoplasm he found the small nuclei, measuring 7–10μ in diameter, unevenly distributed in the cytoplasm, some in the canal system.

Formation of a New Chamber

Some observations were made on the formation of a new chamber. An unusually dense fan-shaped mass of closely set anastomosing pseudopodia make their appearance, radiating out from the terminal apertures of the last chamber of the shell (see Text-fig. 9*a*). Beyond them bundles of ordinary pseudopodia may reach out farther to collect any material that may be available (diatoms, excretory granules, sand, etc.) to make into a 'face-mask', inside which the new chamber will be formed. But the process can go on



Text-fig. 9. *a*, bundle of special pseudopodia emerging from pores of shell just above the foramina and at sides of last chamber where new chamber will lie, 12.30 a.m. Zeiss, $\frac{1}{6}$ in., 4. *b*, detail of new chamber in process of formation. Zeiss binocular, × 300; 11.0 p.m.–midnight. *F.*, cytoplasm in foramen of new chamber, i.e. terminal aperture; *Ps.*1, pseudopodia, gone at 11.30 p.m. leaving cytoplasm with clean outline, ? shell appearing. *Ps.*2, pseudopodia still lively at 11.45 p.m.; *R.P.*, cytoplasmic retral process, 11.30 p.m.; *T.*, tubercles on terminal face of last old chamber which will form part of the new last septum.

without this cover, as in the case of a specially cleaned *Polystomella* under irrigation, and then the details are more clearly seen. Activity usually begins in the late afternoon or early evening, the protoplasm, which may be withdrawn at the time from the last chamber, passing out in the form of pseudopodia which flow through the successive sets of foramina, and also emerge at the fossettes and pores at the sides of the last chamber which will be partly covered by the new one. Some of the special pseudopodia after a time begin to arch over in a reticulum which outlines the cavity of the future chamber, usually about 10 p.m. to midnight, the 'face-mask', when present, being pushed away to the outside. They gradually swell at their bases and merge into one another there, whilst a fluid wells out amongst them and comes to fill up the space they enclose under their extremities with a uniformly granular mass of colourless protoplasm in which the pseudopodial streams fade out and ultimately disappear into the general circulation of the mass (see Text-fig. 9*b*). This comes to have a clear-cut surface, fashioned in the shape of the new cavity even to the retral processes and the projections at the future foramina, as shown in the figure. The longer and more active pseudopodia which normally collect the 'face-mask' material have disappeared, and the short pseudopodia remaining on the surface of the protoplasmic mass now appear stiff, with very sluggish movements. They may come and go, as the shell is deposited on the mass; it is impossible to know how much of their variation under observation is due to the unnatural illumination, etc., but in any case the shell seems to be porous from the beginning, and therefore bathed in protoplasm which lays it down initially and may continue to add to it (and at times to reabsorb it) throughout life. As soon as the surface is available a collection of shining granules may be seen there which gradually form a thin layer of shell. This seems to be laid down in patches like the pieces of a jigsaw puzzle, which unite, losing their separate outlines more or less completely as the shell thickens. After a time the characteristic tubercles are formed on the outside and the keel is laid down at the periphery. During the next day the *Polystomella* remains immobile, while the shell is deposited. There is a flow of brown protoplasm into the now penultimate chamber and sometimes into the base of the new one. Then the colourless mass there becomes vacuolated and may be withdrawn altogether, leaving the new chamber empty for a time, as is usual during the greater part of the life of a *Polystomella*. Pseudopodia emerge, the 'face-mask' is cast off, and the *Polystomella* moves away to begin feeding again some 24 hr. after the emergence of the protoplasmic mass. Although a new chamber may occasionally be formed at other times of day, this usually happens early in the night as described; and therefore it would perhaps be advisable when it is necessary to move *Polystomella* in cultures to do so late in the afternoon, say 5–6 p.m., when there is least chance of spoiling a new chamber with a shell too thin to stand being handled. Even if this is injured, however, or if an irregular chamber is formed for any reason, there may be restitution of the normal shape in the course of one or a few additions if circumstances are favourable.

Earlier observations on the development of new chambers are recorded for *Polystomella*, *Rotalia*, and *Miliola*, by Schultze (1854, p. 30); for *Peneroplis* by Winter (1907, p. 23); for *Quinqueloculina* by Hofker (1930*a*, p. 384); and for *Discorbina* by le Calvez (1938, p. 266). Excepting that I have seen nothing in my *Polystomella* to suggest that a new chamber increases in size after it is provided with a shell (as Schultze maintains), all the accounts agree so far as they coincide.

Schultze (1854) comes to the conclusion that *Polystomella strigilata* (also some Rotalidae) can live for several years, since he was able to keep them alive in captivity for a period of 9 months. From my experience this does not seem likely; I would rather agree with Winter's suggestion of senility ('Peneroplengreise', 1907, p. 19) in individuals which pass the sexual breeding season of their contemporaries without undergoing reproduction. The microspheric form usually appears as a comparatively youthful organism, even when it reproduces; but the megalospheric phase when it is grown up tends to become paler, grows much less, and is generally less active. Although a few may survive the season for sporulation it seems very doubtful that they ever again show great activity. If we regard the two generations as making up the life cycle then, it normally occupies a period of about 2 years.

PART III. SPORULATION. DEVELOPMENT AND EMERGENCE OF THE FLAGELLATE SWARM SPORES,[1] THEIR FORM AND BEHAVIOUR, AND ATTEMPTS TO OBTAIN THEIR FURTHER DEVELOPMENT

Development and Emergence of the Flagellate Swarm Spores

Living Material. Sporulation in *Polystomella* comprises the development and emission by the mature megalospheric stage of very numerous flagellate swarm spores.

All through the winter months it is possible to find undeveloped megalospheric and microspheric individuals down to twenty or thirty chambers or less. These may become pale and shrunken inside their shells during the lean months and many of them probably die. Several small *Polystomella*, somewhat under 1 mm. in diameter, of a good colour, were collected in January 1939 and placed in cultures of *Navicula*, where they fed well and grew new chambers. One reproduced asexually late in April, the other eight included one microspheric (which grew to 1·5 mm. across) and seven megalospheric individuals. But the majority of the megalospheric forms are fully developed by the onset of winter; they will feed and grow a few more chambers when food is available, but from January on more and more of them come to an end in sporulation. The brown colour fades away in a short space of time, a day or two, or sometimes overnight. Meanwhile especially long pseudopodia are put out, often in a long curved bundle which may reach right across an ordinary Petri dish, and large quantities of xanthosomes are shed, so that the pseudopodia may look quite yellow. (This is not, however, a large factor in the loss of colour which is primarily due to an alteration of the chromatophores.)[1] The *Polystomella* walks away from the yellow tracks by means of ordinary colourless pseudopodia. The creature has become very sticky by secretion of a great deal of mucus (see p. 625). The pseudopodia are withdrawn, and the protoplasm in the shell takes on an almost translucent character, while larger or smaller masses of brown excretory granules remaining in the shell often become visible on the under-side—indicating presumably that fission of the cytoplasm has taken place, the heavy, coloured, xanthosomes falling down below the bodies of the developing flagellispores (cf. *Gromia*, Jepps, 1926). A *Polystomella* at this stage is a conspicuous object of a dazzling whiteness which gives it a remarkably plump appearance. The flagellispores usually emerge during the following night, mostly shortly after midnight. They may be seen in amazingly rapid motion inside the shell just before emergence if a thin place can be found and suitably illuminated under the microscope (Zeiss eyepiece no. 4, objective A; × about 100[2]). Presently they all rush out in a great cloud, or they may swim out in a smaller steady stream in the course of several hours, leaving the shell by the foramina and probably by the canal openings also. As a rule shells do not appear to be extensively damaged after sporulation, although some of the usual openings may be enlarged and the little flagellates naturally take advantage of any extra exits that may be available. The shell is left absolutely empty and clean after a good sporulation, apart from small collections of granules, mainly xanthosomes, which may remain in some of the empty chambers; such shells showing brown patches on the side which happens to lie below are recognizable in the collections from the sea for a long time afterwards.

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[1] Known as *zoospores* or *flagellispores* (French) = *flagellospores* (German). The word *flagellula* is to be avoided. It has been criticized as an ill-coined word which does not really mean *a small flagellate* as intended, but *a small flagellum*.

In *Peneroplis* there is said to be a difference in the habit of the two phases; 'Wenn die Agamonten älter werden, hälten sie sich mehr am Boden...die Gamonten steigen höher, was im Reifestadium mir besonders characteristisch schien' (Winter, 1907, p. 20). Nothing of the kind has been detected in *Polystomella*, and it is not possible to say whether there is any special tendency for this foraminiferan to climb up to a higher place before sporulation. Certainly some of those which did so in culture vessels fell off when all the protoplasm retreated inside the shell, although others remained attached by the tough mucous trail of the long cleansing pseudopodia. Lister wrote in his Notebooks (1892–1905, Vol. II), in June 1895, that he had probably failed to find 'zoospores' in 1894 because he had only examined *Polystomella* climbing on the walls of his glass vessels, and these with 'zoospores' would have fallen down when the pseudopodia were withdrawn. Nor is it known exactly when they stop feeding. A few *Polystomella* sporulated in Petri-dish cultures of diatoms, and it was here that the earliest observations were made which gave the clue to the appearance of a sporulating individual. Four healthy brown *Polystomella* (1·3–1·5 mm. across), collected at Drake's Island on 13 January 1939, were cleaned and left in a good culture of *Navicula mutica*, the earth extract medium being replaced by sterile sea water, at a low temperature (10° C. or less). Up to 20 January, no note of any change of colour was made, but on 21 January, one was seen to be lying in the middle of the dish on 'good pasture' with 'many pseudopodia', and 'very white' in colour. Next day the pseudopodia had vanished, but the shell had a light covering of *Navicula*. By 24 January, the temperature being noted as 6° C., it was seen to be empty. On the other hand, one or two other *Polystomella*, which sporulated in this set of cultures later on, had fed and actually grown a few new chambers first.

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[1] It is hoped to describe this more fully in a later part of these studies.

[2] A little care may be necessary to distinguish between this dance of the flagellispores and a somewhat similar commotion in the shell due to an invasion of a more or less decayed *Polystomella* by much smaller motile bacteria.

Many of the details given above were recorded from *Polystomella* taken from the sea, cleaned, and kept in Petri dishes of sea water at various temperatures, and one or two lots kept in the laboratory under other conditions, during the sporulation season.

Although collections had been brought in from Drake's Island at short intervals throughout the earlier winter months, and sometimes kept for a day or two in Petri dishes before being used in experiments with various fixatives, no white *Polystomella* were noticed in them until 15 March. It is probable that they would not have been picked out before their significance was known, and the material was not kept before examination for so long an interval as is usually required for brown *Polystomella* to complete the process of sporulation after capture. It was, however, noted that microspheric forms seemed to be 'very scarce' in the collection of 25 January; and although about 380 *Polystomella* collected on 1 February, some of which proceeded to sporulation in the laboratory 3 days after capture, gave about 6 % of microspheric specimens, a certain amount of selection had in fact been made by picking out those of a large size, so that in all probability the proportion was actually but little, if at all, increased (see p. 635). It is unlikely therefore that very much sporulation had occurred before the end of January. The sporulation season seems to fall during the coldest weeks of the year,[1] possibly reaching its height soon after the vernal rise of temperature sets in. According to Harvey (1928) the lowest winter temperatures for 'inshore water' of the English Channel were recorded (in 1924) about the middle of February, and for 'surface water' about a month later. In 1939 the temperatures posted on Plymouth Hoe from civic observations at the pier fell to their lowest (45–47° F.) during February—so possibly in this year the 'inshore water' where *Polystomella* lives, also passed through its coldest phase somewhat earlier than in 1924. Miss M. F. Mare, who was engaged on planktonic observations at Plymouth in 1939, was kind enough to inform me that the spring diatom increase went steadily forward from about 14 February. Sporulation thus began shortly before the diatom increase was detected, when the *Polystomella* were at the end of their season of scarce food. When it was first observed the hours of daylight had been increasing for a few weeks since the shortest day of winter.

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[1] This is probably what Schaudinn meant when he said 'Mit dem Eintreten der kalten Jahreszeit merkte ich dass viele grosse *Polystomellen* eines Kulturglases nahe daran waren die Flagellosporen zu bilden, d.h.... hatten sogar schon die Sporenkerne gebildet' (1903, p. 500). Le Calvez (1938, p. 216) mentions that he found over 75 % of the *Polystomella crispa* living on the *Lithothamnion* at Banyuls to be microspheric 'pendant les mois froids'.

When it was discovered that a definite season of sporulation was going on, it was decided to seize the opportunity of making a serious attempt to carry the life cycle through this phase, which it was recognized would probably prove one of considerable difficulty if *Polystomella* were found to fall into line with other organisms previously investigated, such as *Gromia oviformis*, for example (Jepps, 1926). The following observations on various factors which might affect the formation of the flagellispores are therefore to be regarded as incidental to the chief object of investigation at the time; and the conclusions on the whole tentative, since so many of the experiments were on a small scale and often not repeated.

When sporulation was first observed in the collected material, the catches were still being brought into the laboratory and washed with filtered outside sea water (see p. 620) which had been standing there, so that the *Polystomella* were warmed up say from about 8° C. in the sea to laboratory temperatures, varying roughly about 13–15° C. It is possible that this stimulated some of them to sporulation, for it occurred in five catches from 25 January to 8 March in a fair proportion (up to 20 %) of the megalospheric *Polystomella*, in each case 3–4 days after capture. In one outstanding experiment 'about 100' brown *Polystomella* were washed well in the laboratory, and then placed in a glass basin containing a substratum of sand and a growth of diatoms, etc., accumulated during several weeks in the sea-water circulation of the aquarium, the temperature remaining between 10 and 11° C. After 3 days some of them were seen to have active flagellispores in their shells, and after 8 days it was found that ninety-seven out of 125 individuals had sporulated, leaving empty shells behind. Of the remainder two seemed dead, one was microspheric and twenty-five were megalospheric. This is the highest proportion of sporulating individuals out of a brown sample that ever occurred in my experiments. I do not think this result is due to the food provided in the substratum; in any case no such increase was observed when *Polystomella* had a few days earlier been placed in a Petri-dish culture of *Navicula* kept in the laboratory; nor to the lower temperature of the circulating water, since a series of samples of the same catch in Petri dishes and in another basin of similar dimensions to the above gave no more than 20 % of sporulation in a total of some 350 *Polystomella* kept at various temperatures between 10 and 15° C. after their washing at laboratory temperature. It may be provisionally attributed to the fact that the water was in circulation in this basin, as it was not in the second basin referred to above, which stood alongside the first and contained similar water.

It would be worth testing this suggestion when the opportunity again arises. Føyn (1936*b*, p. 17) put his *Discorbina* 'unter fliessendes Seewasser zur Schwärmerbildung' with success. It should be noted, however, that a previous lot of *Polystomella* collected on 1 February and treated, so far as is known, in the same way had failed altogether to sporulate in the same basin in a week. The only possible difference seems to be that they might by some chance have missed the stimulus (if it exists) of an appreciable rise of temperature beforehand. A small batch of one dozen megalospheric individuals had given two in sporulation in this basin 3 days after their capture on 25 January, so that this difference is not so likely to be a question of the advancing season.

After 8 March, with a view to avoiding unnatural conditions so far as possible, care was taken not to subject the *Polystomella* to a rise of temperature before sporulation. By getting them early in the day, and washing them in a more or less open antechamber to the constant temperature rooms it was possible to maintain their environment at about 10° C. On 15 March white *Polystomella* were for the first time found in the catch. Of seventeen such individuals thirteen sporulated during the following night, only one or two having a few flagellispores left in their shells by 10 o'clock next morning. The remaining four went translucent, but were fixed without sporulating on 21 March, the filtered outside water having been changed almost daily; only one was found to be microspheric. Of the brown *Polystomella*:

60–70 kept in a Petri dish at 10° C.	Gave 3 sporulating during the night 16/17 March. 1 sporulating during the night 17/18 March.
60–70 kept in a Petri dish at 13° C.	Gave 1 sporulated during 26 hr. from 21 to 22 March.[1]

(These two lots were in filtered outside water changed once a day.)

100 kept in a basin at 11° C.	Gave 1 sporulating during the night 16/17 March.

(This lot was in circulation water. The *Polystomella* which sporulated had gone white by the morning of 16 March.)

It is suggested that the falling off in numbers of sporulating *Polystomella* as compared with the last catch might be due to the omission of the warming up stimulus; as also perhaps their more scattered distribution in time, since on this supposition only those underwent sporulation which would have done so if left in the sea.

By the date of the next collection, 21 March, the season was more advanced and greater numbers sporulated in spite of this omission. It was estimated that about 10 % of the living *Polystomella* in this catch were already white when brought in. The results of experiments with this collection are shown in Table II. The catch was divided into two portions, one of which was placed at approximately each of the temperatures shown in column 1, in filtered outside sea water. After washing the *Polystomella*, the water of those marked + in column 3 was changed daily. The last column shows the total number which had sporulated by 26 March or, in some of the experiments, by 27 March.

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[1] Between 9.30 a.m. on 21 March 1939 and 11.30 a.m. on 22 March 1939.

These experiments suggest that once *Polystomella* has reached the 'translucent' stage the process of sporulation is not as a rule disturbed by fishing it out of the sea, even when it is placed in very much warmer surroundings. It is shown that 'white' *Polystomella* which have not reached so advanced a stage may not sporulate any sooner than those which are still 'brown' when caught. Of these it seems that a considerably smaller number may go on to sporulation if they are kept at the higher temperatures all the time. Something of the kind was hinted at in the few comparable sets of *Polystomella* in the earlier series which were given a warm up on catching and then kept at various temperatures. The small numbers placed in *Navicula* or *Synura* cultures in Petri dishes earlier in the year (see pp. 620 and 636) indicated the same thing. These were washed in the laboratory at room temperature, and then some were placed in a constant temperature room which was cooler. Out of twenty-five known megalospheric forms kept in the laboratory, only two attempted to sporulate; whereas out of twenty-one kept between 10 and 13° C. nine made the attempt. It is interesting to note that the dates of these attempts were spread over the months as shown in Table III, *L* representing cases occurring in the laboratory, and *C* those in the low-temperature room.

TABLE II

° C.	*Polystomella* brought in at 4.15 p.m. 21 March	Water changed	Result	Total sporulated
10	27 'white': 16 'translucent'	0	All finished sporulation before noon on 22 March	16/16
	11 'cream'	0	1 sporulating at noon on 22 March 2 sporulated 22–23 March 1 sporulated at 8.45 p.m. on 24 March 3 sporulated 24–25 March	7/11
	200 'brown': 100	+	Over 50 sporulated during the night 24–25 March, two by 9.30 p.m.	50/100
	100	0	38 sporulated during the night 24–25 March, none by 8.45 p.m.	38/100
14–20	22 'white': 12 'translucent'	0	9 sporulating at 2.30 a.m. on 22 March 2 more early on 22 March	11/12
	10 'cream'	0	3 sporulated 24–25 March	3/10
	200 'brown': 100	+	3 sporulating by 10 p.m. on 24 March	3/100
	100	0	3 sporulated during the night 24–25 March, none by 10 p.m.	3/100

TABLE III

	January	February	March	April	May
	C	*C*	*C*	*L*	*C*
	—	*C*	*C*	*L*	—
	—	—	*C*	*C*	—
	—	—	*C*	—	—
Totals	1	2	4	3	1

The *Polystomella* in any one culture showed no tendency to sporulate simultaneously, and whether the sea water was changed each day or not seemed to make no difference to the number undergoing sporulation.

Table II illustrates the fact that the emission of flagellispores by *Polystomella* collected while still brown most often takes place some 3½ days after their capture, whether they are subsequently kept at laboratory temperatures or in a cold room. Comparatively few individuals sporulated earlier or later. This was found to be true for most of the catches,[1] though for some unknown reason in one or two lots the process went rather faster or a little more slowly. The same thing was found to hold for the few observations it was possible to make at Plymouth in 1940 with *Polystomella* from the 'White Patch' ground. Lister (1895, p. 426) refers to an instance of sporulation 4 days after capture, giving some description of the process. The relative constancy of this interval and the fact that such a large proportion of the catch may sporulate almost simultaneously seem to indicate that something involved in the catching and subsequent treatment of the *Polystomella* has an effect on sporulation in individuals which have reached a certain stage of ripeness,[2] a phenomenon which is probably not peculiar here. Also after the initiation of the process it was found that the disturbance occasioned by examination at an advanced stage might precipitate the emission of the flagellispores. On 19 April a 'cream' *Polystomella* was taken from the catch and isolated in a small Petri dish of earth extract medium at a temperature of about 12° C. At 5.30 p.m. it was still 'cream', and through a rather thin shell it was plainly seen that the protoplasm was undivided. At 9.0 p.m. it was unchanged, and it was then noted that two small rounded masses of protoplasm which had oozed out near the keel of the shell still showed a few brown chromatophores near their centres. There were a few short pseudopodia out. At 11.0 p.m. the *Polystomella* was again put under the microscope. The extruded masses were at once seen to have lost their clear-cut outlines, and their surfaces to be heaving rapidly about as they divided up into smaller masses of irregular shape and then into little spheres with shining inclusions. No brown chromatophores were any longer visible. Flagella broke out before the small spheres (roughly 4μ in diameter) finally became separate. The protoplasm inside the shell was following suit, beginning at the damaged place, and by 2 a.m. the emergence of the flagellispores was in full swing. It has been repeatedly observed that great crowds of these suddenly came out when *Polystomella* which were due to sporulate were put under the microscope for examination, especially during the critical hours about midnight and soon after.[3]

[1] Apart from specimens which become established in cultures.

[2] E.g. it either retards or expedites the process.

[3] A similar experience was had with a large spherical *Gromia oviformis*, about 2 mm. in diameter, collected at Millport on 24 November 1939, and placed in a diatom culture at Glasgow next day. On 4 December there was a whitish film over the brown shell contents; and at about 5 p.m. some protoplasmic masses which had been extruded were divided up to form the uniflagellate swarm spores of this species. There was not much change by 8.0 p.m.

It was noticed quite early in this investigation that a large proportion of the shells were emptied say between 9.0 p.m. and 9 a.m., whether they had been collected before or after the brown colour of their contents had disappeared; so, in order to have a good supply of flagellispores, sporulating *Polystomella* were examined at intervals throughout the night, usually being left in darkness between the examinations. It was thus discovered that emergence of the flagellispores began in the majority at about 2 a.m., often continuing until daybreak, although a few individuals might be active an hour or two before midnight, and a few would not begin until later in the morning of the next day. The only variation in treatment which might possibly have advanced sporulation in my experiments was: (1) changing the sea water earlier in the day, or (2) placing the *Polystomella* in a diatom culture.

Both procedures might have something of the effect of a circulation of the medium, which as we have seen may perhaps facilitate sporulation. Le Calvez (1938, p. 205), after observing that the emission of flagellispores in *Iridia lucida* 'se produit à une heure avancée de la nuit', adds 'J'ai constaté cette sortie nocturne chez presque tous les Foraminifères'. Is the nocturnal habit an indication that darkness itself may also favour the emission of flagellispores? Føyn (1936*b*, p. 17) placed his *Discorbina* 'ins Dunkel' when he desired them to sporulate. On the other hand, flagellispores are quite often seen to be active and emergence to take place during the hours of daylight in the laboratory.

One or two collections taken later than 21 March in 1939 gave no results of special interest here excepting perhaps that of 26 April, which illustrates how the two forms of reproduction are going on simultaneously at this season. The temperature of the sea was recorded as about 10·5° C.; the *Polystomella* were washed at about 12° C. and left at 10° C. Microspheric forms were about as common as megalospheric. On 27 April there were sorted out twelve dark brown individuals showing the microspheric 'figure' (see p. 634) and seventeen paler brown ones. These were placed in well grown diatom cultures, and gave the results shown in Table IV.

TABLE IV

Dark brown 'Microspheric' *Polystomella*		Mid-brown *Polystomella*	Pale brown *Polystomella*
6 kept at 10° C.	6 kept at 13° C.	10 kept at 13° C.	7 kept at 13° C.
Schizogony in 5 by 22 May	Schizogony in 4 by 22 May	Schizogony in 1 by 5 May	Schizogony in 0 by 5 May
1 megalospheric	1 sporulated on 6 May	6–8 sporulated by 5 May	6 sporulated by 5 May
		1 megalospheric	1 megalospheric

One *Polystomella* was observed to sporulate as late as 28 June, but most of the megalospheric individuals surviving so long showed little activity.

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until the *Gromia* was pushed with a dissecting needle, when it suddenly became quite white, and then appeared to start 'steaming'—the 'steam' gradually disappearing in crowds of dispersing flagellispores. The steaming continued at 1.0 a.m. By 9.30 next morning the shell was empty except for the mass of stercomata and xanthosomes left behind by the departed protoplasm, and the Petri dish was swarming with the still active flagellispores.

Lister seems to have made his observations on schizogony in smaller *Polystomella* than those I studied at Plymouth (see p. 634), the smaller sizes given by him for the nuclei in the megalospheric young (see p. 641) being perhaps referable to this fact. The figures he gives for the megalospheric form indicate that they all comprised fewer chambers than my large mature specimens. The highest number of chambers mentioned by Lister is forty-one (1895, table on p. 423, and cf. p. 639 of this paper), and the average number of chambers in twenty-seven reproducing individuals collected March–May 1895 is 30·5 (Notebooks, Vol. 1); whilst here and elsewhere (1895, p. 426) he refers to specimens of seventeen and eighteen chambers respectively which were in course of forming flagellate spores. I also obtained sporulation (but not schizogony) in small specimens, down to about 25–30[1] chambers at any rate. They were noted in the Drake's Island material in March and April 1939; and were much more common in the same months the following year in the collections from 'White Patch'.

I can only suggest that these *Polystomella* happened to come from less favourable grounds than the fine large specimens I have been fortunate enough to be supplied with at Plymouth since 1938. The frequent presence of spines on Lister's small specimens,[2] and the shape of some of his figures are reminiscent of the small *Polystomella* occurring at Millport (see footnote to p. 620) for example, which are probably ill-nourished and subjected to great and disturbing variations in their shallow habitat. An interesting possibility is that the small sporulating individuals may have been produced late in the season of the previous year and so may have missed the time of plentiful food—reproducing nevertheless at about the same date as their better fed and better grown elders of the same generation.

Fixed Material. The first sign of approaching sporulation seen in fixed material is the opening up of extra channels of communication between the chambers, as indicated by Lister (1895, p. 425 and pl. 8, fig. 32*a*; also 1903, p. 71). This condition was found from January onwards in specimens fixed after 24 hr. in the laboratory—which might have brought on the process as suggested on p. 647. It happened that no *Polystomella* was fixed the same day as it was collected until late in March 1939, and by that time freshly caught brown specimens after decalcification showed cytoplasmic bridges growing in from the innermost whorl of chambers to make a connexion with the large central initial chamber (see Text-fig. 10*a*). The same thing was seen in *Polystomella* freshly caught at the 'White Patch' ground at the end of February 1940, when the whole population looked rather pale and starved. In some of these modified specimens the large nucleus has disappeared, and I can see no sign of any nucleus at all, either in whole mounts or in sections.

It is instructive to compare two lots of *Polystomella* collected towards the end of March 1939, i.e. probably at about the height of the sporulation season.

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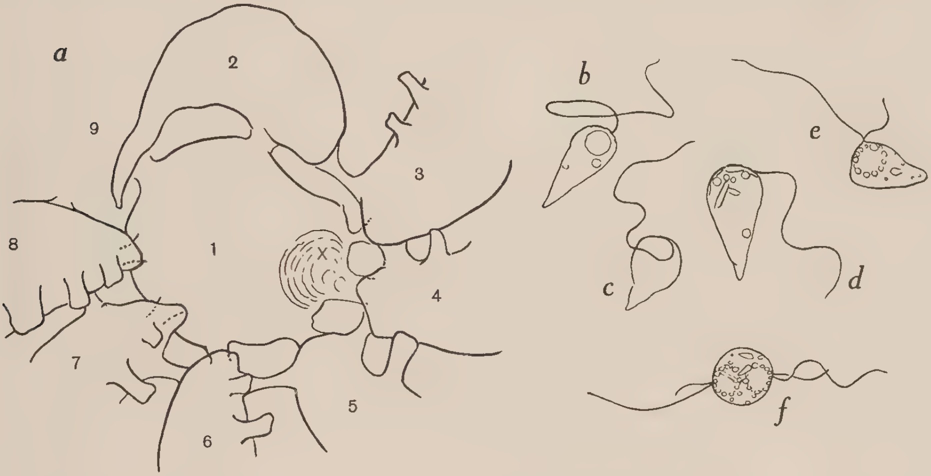
[1] Estimated from the number of chambers exposed in the outermost whorl.

[2] Also seen on a certain number of the smaller specimens from Drake's Island (see p. 641.)

A sample of the catch on 24 March was cleaned and left in a small basin of filtered outside sea water at 9–10° C. Some representative specimens were taken out and fixed each day in Zenker at 40° C. Some of the catch of 30 March, on the other hand, was carefully sorted, at similar temperatures, into five categories by colour, viz. 'dark brown', 'mid-brown', 'pale brown', 'golden', and 'pale cream', which were fixed separately in Zenker as before. From whole mounts and sections of this material a rough time-table of the stages in sporulation may be constructed which agrees quite well with other more casual and fragmentary observations, and was confirmed also by a parallel set of samples from the collection of 30 March, which was allowed to sporulate in Petri dishes. It is of course not to be expected that every sporulating *Polystomella* will adhere rigidly to any time-table.



Text-fig. 10. *a*, sketch of the central cytoplasmic parts of a *Polystomella* collected on 13 January, and fixed 14 January. Zenker at 40° C. Boraxcarmine. Whole mount. 1–8, first eight chambers; 9, position of chamber 9. At *X* is an inrush of cytoplasm, due to fixation, from chamber 4 to the initial chamber (1) through one of the median secondary openings heralding sporulation. *b*, *c*, *d*, sketches illustrating living flagellispores of *P. crispa*. Only one flagellum was seen during life. *e*, the same after exposure to osmic vapour; both flagella are now visible, but there has been some shortening of the body. *f*, a motile sphere, also after osmic vapour, showing a stage in fission.

It is not known how long before flagellispore formation one may find the opening up of the extra channels through the shell—but they appear to increase very fast during the last 48 hr. Not only does the initial chamber acquire median bridges to all the adjacent chambers at their inner ends, but these chambers come to communicate much more freely with one another, involving the canal system, which broadens out, forming wide sinuses in places, as may be clearly shown up when the system is full of xanthosomes on their way out of the shell: the foraminal openings also may become greatly enlarged. A *Polystomella* captured brown at this time of year may begin to look pale on the second day after capture, the brown colour may all be gone by the morning of the third day, and the special extrusion of xanthosomes over. There is often a distinctly yellowish phase, 'golden' in my series, after which the shell contents come to look quite white and ultimately translucent in the course of the third day, immediately before the emergence of the swarm spores during the following night. The large nucleus of the parent megalospheric individual disappears as a rule during the time the brown colour is going. In my preparations it is still present in three out of every four individuals at the 'golden' stage, but in hardly any which were classified as 'white' or 'cream'. It might be mentioned here that the old nucleus seems to be slightly enlarged and rather empty of nucleoli towards the end of its existence; but I have seen no stages in its dissolution. There is apparently an interval during which I can find no nucleus; and then small nuclei, only a few μ across, make their appearance, scattered evenly through the cytoplasm, seeming to increase in numbers until the whole animal is stuffed full of them. One has the impression that they become a little smaller as they increase in numbers as if they were multiplied by some kind of division. Finally, as already described, the cytoplasm undergoes a multiple fission. In sections it begins to look like a fine reticulum with knots in its meshes enclosing one or several of the small nuclei surrounded by darkly staining granules and eosinophil bodies; while, in between, are intensely staining strands which in places form the walls of vacuoles. At these knots rounded bodies gradually concentrate, each of which seems ultimately to undergo two rapid divisions to form the individual flagellispores immediately before they emerge from the shell.

Lister (1895, p. 425) gives an account of his observations on sporulation in *P. crispa*, with illustrations of various stages in pl. 8. I have confirmed the main facts, but I cannot agree with his interpretation of some of the details. The latter are extremely difficult to understand in such a large mass of protoplasm full of granules and other inclusions of various kinds. Fixation also seems unusually imperfect at this stage, owing perhaps to the great amount of mucus which is present. My clearest preparations showing small nuclei were made from *Polystomella* fixed in osmic vapour for 10–15 min., followed by hot corrosive acetic as described on p. 616. I have nothing at present to say about the mode of disappearance of the large nucleus, nor concerning the origin of the small ones. Though I confess I have sometimes not been certain of the presence or absence of the latter, I have never suspected their presence as long as the large nucleus could be seen. They show up at later stages as red or blue spots after picrocarmine or Ehrlich's haematoxylin respectively.

Form and Behaviour of the Swarm Spores

The ultimate divisions giving rise to the flagellispores take place inside the shell as a rule. There is the usual kind of variation in the details of the successive fissions—a coarsely reticular appearance of the cytoplasm having been noted in some cases at a rather late stage; in others, spheres of different dimensions separate off and divide up afterwards to the small swarm spores. These are usually fully formed and very active when they leave the shell, although after emergence figures showing that the last two fissions have not been completed are not infrequently seen (see Text-fig. 10). Pairs of flagellispores are thus not uncommon.

When swimming freely the swarm spores are more or less carrot-shaped bodies, about 6–8 μ long (see Text-fig. 10 *b–d*), proceeding with the blunt end foremost and swinging round in a wide spiral as each little flagellate rotates on its axis under the influence of the rather thick longer flagellum which appears to precede it, but is rarely seen in action owing to its rapid motion of considerable amplitude. This flagellum reaches a length about three times that of the body or even more, and the shorter second flagellum which is invisible during active progression is about body length. (See osmic preparations in Text-fig. 10 *e*, *f*). One gets the impression that it may be carried relatively straight ahead. The whole movement seems well calculated for the sweeping of as large an area as possible by the longer flagellum; as also is the way in which the swarm spores spread out evenly through all the available water, even in the larger vessels, showing no orientation with regard to light, gravity, etc. They swim actively, with very occasional short rests on the bottom, for some hours, say 8–10 under ordinary conditions in the laboratory,[1] and then settle down on the bottom, permanently rounded up in the form of little spheres measuring 4–5 μ across. These gradually fade away, losing their flagella, though their ghosts may be seen for some days, picked out by the shining inclusions which may remain in them. Of these inclusions two kinds have been distinguished: refractile angular bodies of a somewhat crystalline appearance, insoluble in hydrochloric acid and in sodium hydroxide solution, of which half a dozen may be present (Pl. V, fig. 2); and secondly, a small number of less shining discoid bodies which appear to lie flat under the pellicle near the anterior end of the body, soluble in sodium hydroxide, staining blue with Nile blue hydrochloride, and perhaps with methyl green and acetic acid. In osmic acid, although nothing darkens conspicuously, the flagella are well demonstrated. Iodine solutions are useless for showing up the structure.

The swarm spores may go on swimming for half an hour or so under the microscope, especially if uncovered and not too brightly illuminated or overheated. But sooner or later they begin to shorten, and finally round up and become ghostly as described above. In fixed and stained preparations there is a strong tendency to assume the spherical shape, possibly owing to swelling. Figures of selected specimens are given in Pl. V, figs. 2 and 3. The vacuoles, which in some preparations contain eosinophil bodies of various sizes, seem to arise as the swelling comes on.

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[1] But see Exp. 16 (p. 660), when on 31 March 1940 some were still swimming in apparently good form after about 50 hr. in earth extract medium, at 8–10° C., in the dark excepting for three short intervals when they were under microscopic observation.

J. J. Lister (Notebooks, Vol. II, p. 10, dated 4 June 1893[1]) describes a *Polystomella* he had collected the previous day on the shore at Highcliffe (Hants), and which when he crushed it yielded 'zoospores', 'yellow masses' (= excretory granules) and 'algae in pairs' (= foraminal plugs). He did not study the zoospores in detail, stating (1906, p. 8) that he had not even counted the flagella. It seems, however, from other passages in his Notebooks that he believed there were two, and that they were perhaps unequal (see also 1895, p. 426, pl. 8). Other references to these swarm spores in the literature are made first by Schaudinn as already noticed (see pp. 610–646 and Text-fig. 1 D, which is either very diagrammatic or shows a rounded-up form); secondly by Kofoid (1934), who gives additional support to the hypothesis that they are flagellate parasites because Myers (1935*a*) had shown the products of sporulation in another foraminiferan (*Patellina*) to be amoebulae; and thirdly by le Calvez (1938, p. 300), who says in table I that they measure 1·8 × 3 μ, and bear two flagella respectively 12 and 4 μ in length. This last reference is difficult to explain.

Attempts to Trace the further Development of the Swarm Spores

Apart from the casual observation of swarms of the flagellate spores coming from a single *Polystomella* or from two or more individuals, some half-dozen special sets of observations were recorded when the swarm spores were watched under a Greenough binocular microscope for considerable intervals of time immediately after their liberation or a few hours later. Samples of the flagellispores from single individuals were studied swimming freely in watch glasses, and mixtures were made from these in various ways; in Petri dishes also mixed swarm spores from two to four *Polystomella* were watched, at laboratory temperatures or down to 10° C., in filtered outside water or in earth extract medium. In one case the dishes were kept in the dark when not under observation. In no instance was any sign of fusion detected, either amongst these still swimming freely in the water or amongst those which had settled down on the bottom of the dish.

Single flagellispores were seen to tumble about one spot for a while now and then; and sometimes two would tumble about together, thus appearing to be playing around one another, but they always parted company in the end. Finally, as already noted, the swarm spores ceased to move and degenerated, without ever showing the least tendency to become amoeboid.

It might be said that under microscopic observation, even with a Greenough binocular, conditions might be against the performance of syngamy. The series of experiments now to be described, where the swarm spores were not subjected to examination until some time had elapsed, afford no reason to assume that it commonly occurred here either.

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[1] It is not possible to verify my references to Lister's Notebooks at the present time of disturbance as they are stored in a place of comparative safety. The references given are taken from my own notes made at the British Museum some years ago.

SERIES I

This series (Exps. 1–8) was set up with *Polystomella crispa* (P.),[1] from the Drake's Island ground. They were brought into the laboratory and washed with filtered outside sea water (F.O.S.W.) at laboratory temperatures (about 13° C.). Unless otherwise stated they were kept in about 25 c.c. F.O.S.W. in Petri dishes (P-dishes). 'Big basins' measured 9–10 × 4–5 in. and held about 2 l. of medium. Cover-slips (with or without a culture of *Navicula mutica* var. (N.) growing on them) were suspended in the latter on sewing cotton tied to a cross-thread by a fisherman's bend so that they could easily be removed for microscopic examination and replaced: or they were floated on the surface of the medium or placed on the bottom of the dish, sometimes before a culture of N. was grown therein. Earth extract medium (E.) or sterile sea water (S.S.W.) prepared as described in footnote 2 on p. 660 were also used. N. cultures were always grown in E., in P-dishes or in big basins. Sometimes P-dish cultures were placed, uncovered, in big basins of sea water, thus providing a variety of more or less well-stocked feeding grounds.

Experiment 1. Set up during the morning of 4 February 1939. Out of about 100 P. collected 1 February, three shells were found empty, active swarm spores in the dish.[2] Sea water not changed since 2 February. Other P. were removed. On 5 February added 1–2 c.c. of S.S.W. with swarm spores from Exp. 2. Sea water changed[3] for F.O.S.W. at 4.15 p.m. on 4 February, and renewed 5 and 7 February. Temp. about 13° C. Subsequent examinations 7 February (ghosts of flagellispores about 5 μ in diameter seen)[4] and 12 February.

Experiment 2. Set up 11.0 a.m. on 5 February. Two more P. from same lot looked white; washed and left in S.S.W. on 4 February. One showed active swarm spores at 3.30 p.m., both empty next morning. S.S.W. replaced by F.O.S.W., changed 7 February. Temp. about 13° C. Subsequent examinations 7, 12 February.

Experiment 3. Began at 11.30 a.m. on 4 March 1939. In dish of 30 P. collected 28 February and 1 March two found empty. Flagellispores pipetted into N. culture in P-dish at 11.30 a.m., 4.0 p.m., 9.30 p.m., and 5 March at 9.45 a.m. Temp. about 13° C. Subsequent examinations 7, 10, 18 March.

Experiment 4. Set up at 9.45 a.m. on 5 March, from same dish, in which about a dozen P. have now sporulated. Some of the deposit and ten empty shells pipetted into big basin F.O.S.W. with P-dish culture N., and with cover-slips lying on the bottom. On 12 March added 4 P. containing active flagellispores from dish of fifty collected 8 March, two at 2.0 a.m. and two at 4.40 a.m. Cover-slips suspended in basin at 10.0 a.m. Temp. 13° C. throughout. Subsequent examinations 12, 15, 17, 18 March.

Experiment 5. Set up 2.0–5.30 a.m. on 12 March 1939, with 75 P. collected 8 March, and kept at 10° C. in sea water renewed 10 and 11 March 1939. Fourteen P. white and removed to S.S.W. at 2.0 a.m. on 12 March. About ten of these with active swarm spores later transferred to big basin F.O.S.W., with P-dish culture N. and suspended cover-slips. Temp. 10° C. Subsequent examinations 12, 15, 17 March, 10 April.

Experiment 6. Set up at 2.30 a.m. on 12 March 1939, with 85 P. from same collection, kept at 13° C., otherwise treated as in Exp. 5. At 2.30 a.m. on 12 March six with flagellispores were transferred to similar basin. Temp. 13° C. Subsequent examinations 12, 15, 17 March, 17 April.

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[1] See p. 613.

[2] Presumably emerged during the preceding night.

[3] By carefully pouring or pipetting it off, causing as little disturbance as possible to remaining shells, or to flagellispores rounded up on the bottom.

[4] This will not again be mentioned; it is an indication that fairly high magnifications were used in making examinations of material from all the available surfaces in the cultures.

Experiment 7. Dated 10.30 a.m. on 9 March 1939, when 'about 100 P.' from same collection were placed in basin A under the laboratory sea-water circulation system, with good growth of diatoms, etc. (see p. 647). At 4.0 a.m. on 12 March a sample of 4 P. was inspected and all had active swarm spores in shells. On 16 March ninety-seven shells out of 125 were found to be empty. Temp. after first washing 8–11° C. Subsequent examinations 15 March, 23 April.

Experiment 8. At the same time another '100 P.' were placed in basin B of filtered circulation sea water, with N. cultures in P-dishes on bottom. On 12 March 1939 there were seventeen empty shells out of 112. Temp. as in Exp. 7. Subsequent examinations 15, 17, 18 March.

SERIES II

In this series (Exps. 9–15) the *Polystomella* were not warmed before sporulation excepting where stated. Collected from Drake's Island ground.

Experiment 9. Set up at 2.0 a.m. on 22 March 1939.

(1) Three 'translucent' P. from dish of twenty-two white P., collected 21 March, washed at about 20° C., and kept at 14° C., were transferred to P-dish culture N. at 2.0 a.m., with active flagellispores emerging. Shells almost empty at 4.0 a.m. Temp. 14° C. Subsequent examinations 11.0 a.m. on 22 March, 9 April.

(2) Five similar P. kept separately in watch glasses A–E of F.O.S.W., in damp chamber. Examined 6.0 a.m., 11.0 a.m., 9.45 p.m. (no movement now) on 22 March and on 23 March.

(3) Mixed swarm spores from watch glasses A+B+C+D in P-dish culture N. Examined 11.0 a.m. on 22 March, 9 April.

(4) Mixed swarm spores from watch glasses A+B, B+D, A+D, in three separate watch-glasses in damp chamber. Examined 6.0 a.m., 11.0 a.m., 9.45 p.m., on 22 March.

Experiment 10. Set up at 3.30 a.m. on 22 March 1939. Three translucent P. from stock dish of twenty-seven white P. similar to last, but kept throughout at 10° C., with active flagellispores in shells, put into P-dish culture N. At 4.0 a.m. added some flagellispores from eight more P. which had become active in stock dish. Examined 11.0 a.m. on 22 March, 9 April.

Experiment 11. Set up at 8.30 p.m. on 27 March 1939. Out of 'about 100 P.' collected 24 March 1939, and kept at 9–10° C. in small basin, four emitting flagellispores were placed in 400 c.c. F.O.S.W. in flask in which there was a little brown algal growth.

(1) Some of this suspension was kept in P-dish F.O.S.W. at 9–10° C. Examined 11.0 p.m. on 27 March and on 28, 29, 31 March, 9 April.

(2) At 10.15 p.m. on 27 March 1939 flask emptied into big basin with two P-dish cultures N., and suspended cover-slips. Kept at 9–10° C. Subsequent examinations 28, 29, 31 March, 10, 16 April. Temp. rose to 20° C., one day[1] and fell again to 11–13° C. From 22 April kept at 13° C. See Exp. 15 for further examinations.

Experiment 12. Dated 9.50 a.m. on 1 April 1939. Two P. out of dish of three pale brown P., collected 30 March, emptied overnight. Temp. 9–10° C. throughout. Subsequent examinations 3, 4 April.

Experiment 13. Set up in the morning of 3 April 1939. One P. sporulated overnight in each of two dishes containing three and thirteen mid-brown P. respectively, collected 30 March. Swarm spores were mixed:

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[1] The temperature varied a good deal in this experiment owing to the late spring sunshine on a glass roof, and it was well above that of the sea at the time, viz. 10–11° C.

(1) '1 volume' from each, in each of four watch-glasses in damp chamber. Temp. 9–10° C. Examined 1.0 p.m. on 3 April and on 4 April.

(2) '2 volumes' from each, into P-dish culture N. Kept at 9–10° C. Examined 3.30 p.m. on 3 April (but very small organisms would hardly have been distinguished in the body of the culture), 9, 16 April. Temp. rose to 20° C.[1]

(3) '2 volumes' from each, in P-dish F.O.S.W. Kept at 9–10° C. Examined 1.0 p.m. on 3 March and on 4, 5, 9 April.

(4) '2 volumes' from each, to each other in original dishes. Kept at 9–10° C., behind a screen to shade from direct light from window. Examined 1.0 p.m., 8.30 p.m. on 3 April and on 4, 5, 9 April.

Cover-slips were floated on top of medium in P-dishes (2), (3), (4).

Experiment 14. Set up at 2.10 a.m. on 20 April 1939. Pale P., collected 19 April, kept in separate P-dishes E., under black paper, at 12–14° C.[1] Three had swarm spores emerging at 2.10 a.m. on 20 April, viz. dishes A, B, and 2. Examined these dishes 5.0 a.m., 11.0 a.m., 9.30 p.m., on 20 April and on 21 April.

(1) The three shells and some emerged swarm spores from A+B+2 pipetted into P-dish E., and then to big basin E. with P-dish culture of N., suspended and floating cover-slips. Kept about 12° C. Examined 21 April, 6 May (E. replaced with F.O.S.W. on 31 May, and basin used as pasture for freshly caught P.), 24 June.

(2) Swarm spores from A+B+2 pipetted into P-dish E, kept under black paper, at about 12·5° C. Examined 5.0 a.m., 6.0 a.m., 11.0 a.m., 6.0 p.m. on 20 April and on 21, 22, 23, 24 (changed E.), 26 April.

(3) Swarm spores from A+B+2 pipetted into P-dish culture N. in E. Temp. about 12·5° C. Examined 6.0 p.m. on 20 April (obscured by *Bodo*), and on 30 April.

(4) Mixed swarm spores from A+2, B+2, A+B, respectively on slides at 2.50 a.m. Temp. 12·5° C. Examined 3.0 a.m., 5.0 a.m., on 20 April (flagellispores rounded up and motionless).

(5) Mixed swarm spores at 11.0 a.m. from A+B+2 in a fresh P-dish E. Temp. 12·5° C. Examined 6.0 p.m. on 20 April and on 21, 22 (changed E.), 23, 24 (changed E.), 26 April.

(6) Mixed swarm spores at 11.0 a.m. from A+B, A+2, B+2, A+B+2, respectively in four separate watch glasses in damp chamber. Temp. 12–14° C. Examined 9.0 p.m. on 20 April and on 21 April.

Experiment 15. Dated 9.30 a.m. on 28 April 1939. Out of seventeen rather pale P., collected 26 April, and put into big basin used in Exp. 11, after removing four empty shells, on 27 April, four were found empty.[2] Temp. 13° C. Subsequent examinations 6, 22 May.

SERIES III

In this series (Exps. 16–18) the *Polystomella* were collected at the 'White Patch' ground.

Experiment 16. Set up at 6.0 a.m. on 29 March 1940. Room darkened 28 March to 12.30 p.m. on 30 March. From seventeen white P., of various sizes, collected 28 March, washed F.O.S.W. at 5·0° C., kept in two P-dishes E. (containing 4 and 13 P. respectively) at 8–10° C., flagellispores began to emerge at 1.30 a.m. on 29 March.

(1) At 6.0 a.m. 13 P., some from each P-dish, with swarm spores, put into N. culture grown in big basin, cover-slips suspended, kept at 9·0° C. Examined 1.0 p.m., on 29 March (seven shells quite empty), and on 31 March, 4 April. And see Exp. 17.

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[1] The temperature varied a good deal in this experiment owing to the late spring sunshine on a glass roof, and it was well above that of the sea at the time, viz. 10–11° C.

[2] Presumably emptied overnight.

(2) P-dish of E. which had contained thirteen white P. and had many flagellispores, kept at 8–10° C. Examined 6.30 a.m., 11.0 a.m. on 29 March and on 30, 31 March (a few carrot-shaped flagellispores still swimming),[1] 4, 10 April.

(3) P-dish which had contained four white P. and had few swarm spores, kept at 8–10° C. Examined 6.30 a.m., 11.0 a.m. on 29 March and on 30, 31 March.

Experiment 17. Set up at 11.30 p.m. on 8 April 1940. Out of about thirty brown P. collected 5 April, and kept in two P-dish cultures N., i.e. food available, at 8–10° C., three from one dish and one from the other with active swarm spores put into watch-glass, and half an hour later transferred to big basin used in Exp. 16 (1). Temp. 8–10° C. Subsequent examinations 11 April, 18 July (temp. now 13·5° C.).

Experiment 18. Dated noon on 9 April 1940. In dish of about thirty brown P., from same collection, kept in E. with no food, three small P., i.e. not over 1 mm. in diameter, emptied since 3.30 a.m. Temp. 8–10° C. Examined 10 (found small shell, see p. 610), 11 (removed other P. and put in a little N. culture), 12 April.

The series of experiments is, as will be seen, incomplete, but a fair range of conditions is covered. No further development was obtained in any, excepting perhaps the last which produced the small shell described on p. 610 (see Text-fig. 2). Nor was any later stage ever seen in the cultures of *Polystomella* started in January 1939 in diatom cultures (p. 649), where, however, it happened that there never were swarm spores from more than a single *Polystomella* at any one time. It was estimated that a single parent *Polystomella* of moderate size might produce swarm spores to the order of some 500,000; and when one considers the relatively small number of microspheric individuals developed in nature from the large proportion of megalospheric *Polystomella* in the ordinary population, which themselves originate in broods of less than 200 from each of the former, one should perhaps not be surprised at a very low measure of success in experimental cultures, even if it takes two swarm spores to produce one microspheric animal. Their natural rarity does seem to suggest some special difficulty in their development; and perhaps one may conclude from these experiments that it is probably not simply any difficulty in one swarm spore making contact with another. It might be that union can only occur between very special pairs of flagellispores, or that some very special circumstance is necessary for their further development, with or without fusion. It may be that this difficulty is not confined to *Polystomella* (see p. 611, and cf. Rhumbler, 1909, p. 325). It would therefore be well worth while to make further attempts to discover what it is.

In the meantime attention might be drawn to the following points:

(1) *Medium.* Gross (1934) reports a striking success in the culture and induction of syngamy amongst the flagellate gametes of *Noctiluca* (Cystoflagellata) in earth extract medium, a success he had failed to achieve in sea water. In my experiments sea water of various kinds[2] was used; and also earth

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[1] After an unusually long interval, since all shells had been removed at 6.0 a.m. on 29 March (see p. 655).

[2] Viz. sterile sea water, i.e. boiled and kept in the laboratory for at least a month before use; outside sea water (see p. 620), filtered through a single filter paper; and sea water circulating in the aquarium tanks, which, however, since the time of Lister (1903) is known not to be satisfactory for certain stages in the development of *Polystomella*.

extract medium, the only (doubtful) success being obtained when the latter was used throughout sporulation and subsequently (Exp. 18). In this case no diatoms were present until late in the experiment, which almost certainly means that the *p*H was about 8·0. In the *p*H determinations made now and then values above 8·5 were never obtained, even in Petri-dish cultures of *Navicula* so long as they remained healthy. Nevertheless, the *p*H was always a little higher in the presence of diatoms, and in view of the results of Gross (1934) even this small rise may make a difference. The unusual survival of flagellispores noted in Exp. 16 occurred in similar circumstances.

(2) *Food.* It is hardly possible that this is important during sporulation and the earliest succeeding stages—excepting perhaps in so far as its presence affects the *p*H. In the experiments there are several instances where the flagellispores had hours together in plain sea water or earth extract medium beforehand, even if they were later placed with diatom cultures. These it was hoped would have supplied a sufficiency of suitable food of various sizes for the earliest stages, beginning with such small organisms as the bacterial growths which were always present. In other cases sporulation was at least completed in a diatom culture.

(3) *Temperature.* The experiments were carried out at various temperatures, Exp. 18 at the lowest of all (under 10° C.). It is believed that sporulation is best carried out at such temperatures (see p. 649); and arguing from natural conditions in the sea it may be expected that the early subsequent development is at any rate possible at similar low temperatures. It does not follow, however, that it is readiest under these conditions, as is indicated by the work of Maclagan (1932) on *Smynthurus* (Collembola), in which it was shown that, though this insect lays the largest number of eggs at about 7° C., development proceeds best at about 16° C.

(4) *Concentration of Swarm Spores.* This may be important in more ways than one. Apart from the well-known facts that any kind of development may be rendered abnormal, or may cease altogether, when the organisms are too thick on the ground, and that if it is a case of syngamy a certain minimum concentration is on the other hand necessary, it has been suggested that facilitating substances may be passed into the medium by one or both of the partners in such fusion (see, e.g., *Nature*, Vol. 143, 17 June 1939, p. 1036, referring to the work of Moewus, Kuhn and others). Such substances must be present in the medium in certain concentrations.

No attempt was made to count the flagellispores per unit volume, but a good range of concentrations was in fact used in the course of the experiments. Renewing the medium at various stages might also have an effect in this respect, and here again there was considerable variation in the procedure.

(5) *Light.* Light as is well known has many unexplained affects on living organisms. Reference to some of these is made in *Nature* (loc. cit.), where the liberation of the special sensitizing substances in the presence or absence of light is noticed in connexion with syngamy in *Chlamydomonas* (Phytomonad-

ina) and in fishes respectively. No special arrangements with regard to light were made excepting where mentioned in Exps. 13, 14, 16.

(6) *Maturing of the Flagellispores.* This might have been affected by the disturbance suffered by the *Polystomella* taken from the sea and subjected to experiment. Possible effects would perhaps have been varied by the use of flagellispores produced: (*a*) During the night following capture (Exps. 9, 10, 14, 16), (*b*) two to four days after capture (Exps. 1–8, 11, 12, 13, 15, 17, 18); or (*c*) by *Polystomella* which had been for some time in culture. As already explained swarm spores from more than one such *Polystomella* were never available at any one time, so that syngamy might here have been out of the question for a different reason.

It might also be that the flagellispores are only capable of syngamy during a limited period of time after they are liberated. Some attempt to circumvent such a difficulty was made by mixing the swarm spores at various intervals after emergence, and in other experiments allowing them actually to come out more or less simultaneously from two or more *Polystomella* previously placed together in the same dish.

(7) *Selective Mating of the Presumptive Gametes.* It has been established in some of the lower vegetable organisms amongst the algae and fungi that syngamy only takes place between the products of certain pairs of parents, though these may not differ in any visible characteristics. American investigators have recently claimed that there are distinct 'mating types' amongst the Ciliata, only some of which are interfertile (see Jennings, 1939). Whatever the explanation of this, if similar barriers exist in *Polystomella*, it was hoped that they might have been overcome by using as many parents as possible in the experiments. Although only two or three were usually available, flagellispores from 'about a dozen' (Exp. 4), 'about ten' (Exp. 5), six (Exp. 6), and seven (Exp. 16), were used in others. In Exps. 9 and 14 mixed flagellispores from different individual pairs were observed, and no difference in behaviour noted.

Because these experiments are only a beginning, and have not as yet given any positive result, it is thought worth while to describe them in some detail, in the belief that when this line of inquiry is resumed, perhaps by another investigator, they may provide a starting point a little further on than was available in 1938.

SUMMARY

Part I. After a brief statement of the present unsatisfactory position regarding the life history of *Polystomella crispa*, an account is given of experiments with various fixatives on *P. crispa*, collected near Drake's Island, Plymouth. A method is described for the cultivation of *Polystomella* in diatom cultures, especially with *Navicula mutica* var., with which a flagellate (*Bodo* sp.) was constantly present. The identification of individual Foraminifera, and determination of growth, were often possible by some irregularity in the shell, or by noting the attachment of one or more stalked egg cocoons of an unknown

turbellarian worm. The feeding of *Polystomella* on diatoms seized by the pseudopodia outside the shell is described; the structures previously taken for ingested algal cells inside the shell are shown to be foraminal plugs ('bouchons' of le Calvez) which are discarded from time to time and thrown out of the shell along with the excretory granules (xanthosomes), mostly via the canal system.

Part II. Notes are given on the microspheric form and its reproduction, through the spring and summer, by schizogony. The rearing of two broods in laboratory cultures is described, with an account of the formation of a new chamber to the shell. It is concluded that a complete life cycle consisting of one microspheric and one megalospheric phase occupies a period of about two years.

Part III. Sporulation of the megalospheric form is described as seen during life; and various factors are discussed which might affect the process. The relatively small size of the reproducing *Polystomella* with which Lister worked is ascribed to their having lived under less favourable conditions. From a study of stained preparations, certain details are added concerning the opening up of the shell, presumably to facilitate the eventual escape of the flagellate swarm spores; also concerning the accompanying cytoplasmic and nuclear changes. Some account is given of the form and structure of the swarm spores, both alive and in permanent preparations. Experiments are described which constitute an attempt to carry the life cycle beyond the stage of sporulation. They are so far unsuccessful, with one possible exception, under all the various experimental conditions which are briefly discussed in their turn.

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EXPLANATION OF PLATES IV–V

I am indebted to the Carnegie Trust for a grant towards the cost of the coloured figures in Plates IV and V.

Plate IV

Painted by Miss Cecily Brown Kelly.

Fig. 1. A living *Polystomella crispa* from Plymouth.

Fig. 2. Empty shell of same to show terminal face of last chamber. *Ch.*, chambers of the shell, the cavities of which are in communication by the foramina; *for.*, foramina in front of last chamber, which form the terminal apertures of the shell; *foss.*, fossette (d'Orbigny); *K.*, keel of the shell; *ps.* pseudopodia arising from protoplasm inside and outside the shell; *ret.pr.*, ridges over the retral processes of the chambers, between the fossettes; *sep.*, ridges over the septa between the chambers; *ter.ap.*, terminal apertures of shell; *umb.*, umbo.

Plate V

Fig. 1. *Bodo* sp. *Corr.ac.*, iron haematoxylin. This fixative gave better results with this flagellate than did Schaudinn's mixture, Bouin, or osmic vapour and alcohol. A fair number of nuclei in the preparation show the large karyosome and peripheral granules characteristic of the genus, and in many cases the kinetoplast does not stain uniformly, sometimes appearing as a thick-walled empty sack, especially after hot Schaudinn. *N.*, nucleus; *K.*, kinetoplast.

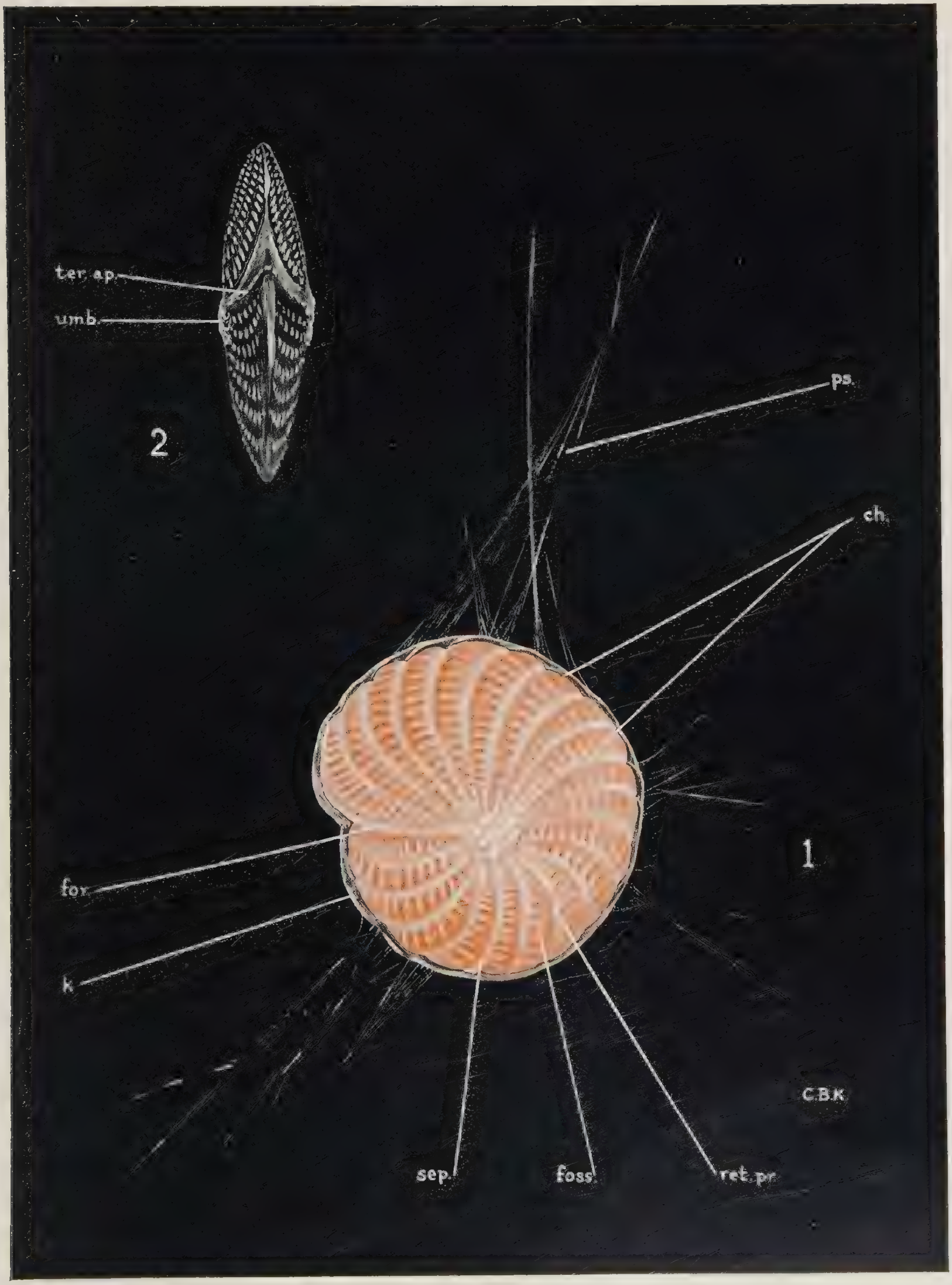
Figs. 2, 3. Flagellispores of *Polystomella crispa* stained with iron haematoxylin and eosin, after fixation in osmic vapour and alcohol and in Schaudinn's fluid respectively. In Fig. 3 the longer flagellum was about five times as long as the body. *cryst.*, refractile inclusion; *N.*, nucleus; *eos.*, eosinophil body in vacuole.

Figs. 4–6. Illustrating three successive stages in the feeding of *Polystomella*, as described in the text on p. 629.

Fig. 7. Part of Fig. 5 at a higher magnification, showing undigested diatoms, and excretory granules (xanthosomes).

Fig. 8. Part of a similar cast feeding cyst showing frustules of a naviculoid diatom and excretory granules.

Figs. 9, 10, 11. Pseudopodia of *Polystomella*. From film made by A. G. Lowndes. Zeiss obj. A, C, D, resp., eyepiece ×10. The granules are well shown in Fig. 11, anastomoses in Fig. 9 and 'webs' in Fig. 10. The black segments at the margins are the edges of the shells.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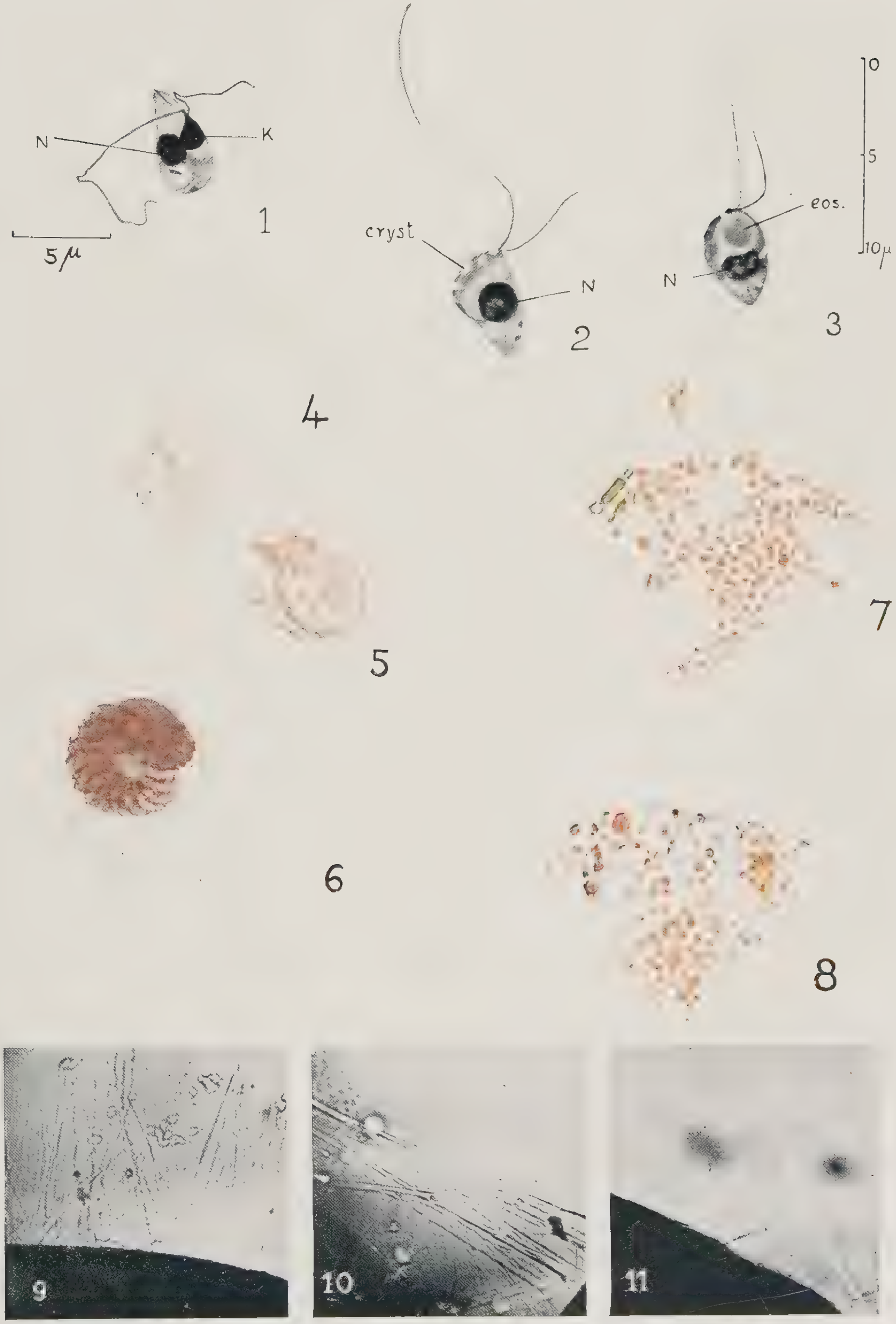
Polystomella crispa L.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Polystomella crispa L.

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

The Mechanism of the Semicircular Canal. A Study of the Responses of Single-fibre Preparations to Angular Accelerations and to Rotation at Constant Speed

By **O. Lowenstein and A. Sand**

Proc. Roy. Soc., B, Vol. 129, 1940, *pp.* 256–75

Single-fibre responses from the horizontal ampulla of the isolated labyrinth of *Raja* have been recorded by the oscillographic method with the aim of a quantitative analysis of the responses of this sense organ. The rate of increase or decrease of discharge above or below the level of a spontaneous resting rhythm is a linear function of the rate of angular acceleration. The threshold acceleration first producing a noticeable response from the organ is in the region of 3° per sec.^2

During prolonged rotation of constant speed the frequency of discharge, having attained a maximum or minimum according to the nature of the initial acceleration, gradually returns to the spontaneous value over a period of about 20–30 sec. These results when interpreted in terms of the physical properties of the cupula terminalis account adequately for the time relations of the rotatory nystagmus and after-nystagmus of the eyes. O.L.

The Individual and Integrated Activity of the Semicircular Canals of the Elasmobranch Labyrinth

By **O. Lowenstein and A. Sand**

J. Physiol., Vol. 99, 1940, *pp.* 89–101

The individual responses of the ampullary end organs in the three semicircular canals of the isolated labyrinth of *Raja* have been investigated by the oscillographic method, with the aim of ascertaining the mode of collaboration of individual sense endings during rotation and tilting about the vertical axis and the longitudinal and transverse horizontal axes. When the labyrinth is at rest there is a spontaneous discharge of impulses from each ampulla which is increased or inhibited when the labyrinth is rotated or tilted. The horizontal canals respond to rotation about the vertical axis, but not to rotation about the two horizontal axes, whereas the anterior and posterior vertical canals respond to rotation about all three axes. The six canals of the two labyrinths collaborate in pairs exhibiting lateral, transverse and diagonal synergy according to the nature of the rotation. The integrated action of the canals during rotation about the three axes was analysed in relation to the eye-muscle reflexes evoked by these rotations. O.L.

OSMOTIC RELATIONS OF SOME METAZOAN PARASITES

By **N. Kesava Panikkar and Nora G. Sproston**

Parasitology, Vol. XXXIII, 1941, *pp.* 214–23

The osmotic behaviour of three parasites in normal and experimental media has been studied with a view to understanding the relationship with their hosts. *Angusticaecum* sp., a nematode from the intestine of the tortoise, is hypertonic in media of very low concentrations (1·1–1·3 % NaCl in tap water), but becomes isotonic in sea water and slightly hypertonic in 50 % sea water. Ligaturing experiments show that its cuticle is permeable to water and probably to salts. *Lernaeocera branchialis*, a blood-feeding copepod from *Gadus* spp., is hypotonic to the surrounding sea water so long as it remains attached to its host, its blood showing an osmotic pressure equivalent to 2·0–2·8 % NaCl. Isotonicity with the medium is established when the parasite is excised and kept alive. Hypotonicity of *Lernaeocera* is probably caused by the low osmotic pressure of the blood of its host (1·443 % NaCl in *Gadus pollachius*), to which it is permanently attached. *Bopyrus squillarum*, a blood-sucking isopod from *Leander serratus*, is isotonic or slightly hypotonic to sea water, the tendency towards hypotonicity being probably the result of the hypotonic nature of the blood of the host. The osmotic properties of *Lernaeocera* and *Bopyrus* would suggest their ability to survive in dilute sea water, a fact which is supported by their occurrence on hosts living in inshore or estuarine habitats. N.K.P. and N.G.S.

A COMPARATIVE STUDY OF THE EFFECTS OF IONS ON WHOLE NERVE AND ISOLATED SINGLE NERVE FIBRE PREPARATIONS OF CRUSTACEAN NEUROMUSCULAR SYSTEMS

By **Talbot Howe Waterman**

J. Cell. Comp. Physiol., Vol. 18, 1941, *pp.* 109–26

A comparative study of the effects of ions on the perfused neuromuscular system of three decapod crustaceans has been made. The contractile responses of the flexor dactyl muscle to stimulation of the whole leg nerve (in *Panulirus*, *Maia*, and *Cambarus*) and to stimulation of isolated single motor nerve fibres (in *Cambarus*) have been isometrically recorded. The tension developed in response to brief tetanizing stimulation varied inversely with the amount of magnesium in the perfusion fluid, being greatest when none of this ion was present and approaching zero when four to five times the normal amount were perfused.

Changes in perfusion fluid calcium content usually gave rather irregular results, but some single nerve fibre experiments suggested that this ion had qualitatively different effects on the slow and the fast neuromuscular systems. Increased potassium led to decreased contractile responses in whole nerve preparations and single nerve fibre preparations of the slow closer system, but to markedly increased contractile responses in single nerve fibre preparations of the fast closer system. Exactly comparable results were found when both the slow and the fast motor closer motor fibres were prepared and alternately stimulated in the same nerve muscle preparation. Evidence is cited suggesting that these potassium effects occurred at the myoneural junction in some of the steps involved in neuromuscular transmission.

T.H.W.

THE ACTION OF POTASSIUM ON ECHINODERM, MOLLUSCAN AND CRUSTACEAN MUSCLE

By G. P. Wells

J. Exp. Biol., Vol. 18, 1942, *pp.* 213–22

The effects of various potassium concentrations on the cloacal complex of *Cucumaria elongata*, and on the hearts of *Aplysia punctata*, *Helix pomatia* and *Carcinus maenas* are described. It is pointed out that there exists a fundamental similarity between many, and perhaps all, types of rhythmic muscle, as regards their responses to changes in potassium concentration.

Data on calcium : potassium antagonism and ammonium : potassium parallelism in the *Helix* heart are also presented; in connexion with the former, there is a brief discussion of the physiological differences between marine and other animals.

G.P.W.

Journ. Marine Biol. Assoc., Vol. xxv, p. 671.

E. J. Allen.

EDGAR JOHNSON ALLEN

1866–1942

The late Dr E. J. Allen was the son of the Rev. Richard Allen, a Wesleyan minister and as such liable to change the sphere of his work every few years. For this reason E. J. Allen passed his childhood in Cornwall, went to school in Exeter, and lived during his late boyhood in the Worth Valley, going into Leeds to attend the Yorkshire College (now University of Leeds); his education was continued by the University of Berlin, an interval of teaching in Antigua, and University College, London, followed by a Royal Society research studentship at the Plymouth Laboratory. Excepting Antigua, every one of these epochs claimed in him its own loyalties: a few years ago he seriously considered going west to Cornwall to end his life where he first remembered it; he much admired Franz Eilhard Schulze, who taught him at Berlin, and W. F. R. Weldon, his Professor of Zoology at University College, yet kept a lively gratitude for what he had learned from Professor Miall at Leeds.

To this very varying environment during the first third of his life we may attribute through the next half-century much of his universal power of sympathy and contact with all sorts and conditions of men. Unselfish by nature, he had the example of a singularly wide-minded father; then bred as a boy in Cornwall, Devon, and West Riding, as a man among London University students and Berlin University students, with the West Indian interlude—there was no class or nation who could not feel an understanding with Allen.

I first heard of him from Weldon—probably in 1891. 'I say! I've got a new sort of student at U.C. now—name of Allen. I gave them the classification of the Crustacea, and at the next lecture he brought up to show me, in case anything interested me, a collection of Crustacea he had made in Antigua, showing some he found rather difficult to fit in!' Weldon had always a great regard and very high esteem for Allen: to this I should attribute Allen's whole connection with Plymouth, from his first visit for research in 1892 to his appointment as Director and Hon. Secretary in 1895.

We met in the Laboratory at the end of 1893. Bles (Director), Cunningham (Naturalist) and Garstang (Assistant Naturalist) were the Staff naturalists; Allen, Hickson, and myself, were the visiting naturalists. Our laboratory assistant was a young man of 22 named Joseph, who was monarch of all he surveyed on the laboratory floor, and was not quite conscious of his own limitations. Roach was our collector and fisherman, an admirable man at that time, who could do anything with the 18 ft. open boat, *Anton Dohrn*, which formed our fleet, always ably seconded by 'Bill', who, as W. Searle, now numbers 50 years of friendship among biologists working at Plymouth.

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Outside his work on the nervous system of the embryonic lobster, Allen had started an experiment on filtering water from the circulation through Berkefeld porcelain filters. It was unsuccessful (he had not coped with the difficulties of keeping the filtrate sterile), but it was his first attempt in 1894 to deal with the problem, which he solved in 1910, of preparing sea water in which delicate larvae could be reared.

He lodged in Windsor Lane (ground floor on the right hand as you go down from the Hoe, I think the second door). We led quiet and studious lives, and he had a reserve or a shyness which I found difficult to penetrate at that time; looking back, I think a natural shyness was then accentuated as the result of eight years of uphill life in which he had not found the world gentle. Outside work, our greatest excitement was when J. T. Cunningham and his wife asked us to Sunday supper—hospitality most pleasantly given and most gratefully received by two bachelors in lodgings; Mrs Cunningham was a sweet-natured woman. Mrs Bles had a serious illness on her arrival at Plymouth, so that we did not visit the Director's house until later in the year. Hickson, in the laboratory compartment on my left, was the best of neighbours; Garstang was not only a mine of information on the fauna of the Sound but was a source of cheerfulness and good companionship to us all.

In the autumn of 1894 Bles gave up the contest, in which he had struggled valiantly and well, and Allen was appointed Director in his place; a place that had now been filled by four Directors in eight years. But Weldon had already confided in me that he thought the constitution of the Association was more to blame for these rapid changes than defective qualifications in the four Directors. The Hon. Secretary was in the habit of making criticisms to the Director and bringing before Council any matters in the conduct of the Laboratory which he considered desirable to be discussed with the Director. In other words it was the Secretary's duty to scratch the Director's back: and E. Ray Lankester scratched Heape's back good and hard, while G. H. Fowler did the same for Calderwood and Bles (G. C. Bourne could look after his own broad back). Remembering also that Allen was Weldon's pupil—and remembering all he did for me, who had also been his pupil—I attribute to Weldon salvation of the M.B.A. by the revolutionary change making Allen the first Director also appointed Honorary Secretary.

So, in January 1895, aged 28, Allen took up his duties, with behind him a discouraging Bluebeard's cupboard of ex-Directors, yet holding one immense essential advantage over them: that he had only to deal with the Council and the President, not with an official critic busy in preparing for them a synopsis of his three months' errors.

This was an immense advantage, but even so the prospect was enough to make even a bold man shrink. We had a fine well-designed marine laboratory, with comfortable 'tables' for eleven workers and two or three extra rooms. In December of 1893, when Garstang, Allen and Hickson had gone for Christmas holidays, for a week or two Cunningham worked on the north side of the Laboratory and I on the south side. The Director left his wife's sick bed to pay us each a diurnal visit, and Cunningham and I kept each other company for the rest of the day.

Financial stringency was dire. Income all in was £1950 (of which £1000 was granted annually by the government and £400 by the Company of Fishmongers). The salaries we paid were beggarly: £200 a year and a house to the Director, £250 to the Naturalist, £150 to the Assistant Naturalist, if there was one, and £350 in all to pay fishermen, laboratory attendant, engineer and clerk; yet Allen put the necessary minimum expenditure at £1800 without any assistant naturalist. There was the cavern of a Laboratory, weathertight and with pumps going, in which the Director, the Naturalist, and the Assistant Naturalist made their researches while a dozen naturalists visited them during the year, most of them for only a few weeks in the summer.

I came down to Plymouth in July 1895, when Allen had been six months in the saddle. July and December at the Laboratory were very different things and I found a most pleasant company: W. F. R. Weldon, W. Garstang, A. Bethe (Munich), J. D. Gilchrist, T. H. Riches and W. I. Beaumont, with T. V. Hodgson, newly appointed as Director's Assistant, and three undergraduates. Weldon's presence was a great pleasure to Allen and me. He was rearing 500 young crabs through a moult, to ascertain how far deviation from the normal was the same for the old and new carapaces of each crab; Allen had rigged him a perfect apparatus—a sloping rack of shelves under a corrugated iron pent house over the open-air tank at the back of the aquarium. On the shelves were 500 glass jars, each supplied with a jet of sea water and containing a live crab. (It looked so like a display at a grocer's shop that the irreverent referred to it as 'Gubbins & Co.') Every day each crab was fed with fresh fish, and the cutting of fillets of young soles the moment they were dead was hard manual labour. I think Weldon's visit lasted out August.

Garstang was no longer on the staff, but a Fellow and Lecturer of Lincoln College, Oxford. He was only down for five weeks, working at Tunicates, but also, as in a previous visit in March, was conducting a most important experiment: the first of 'Garstang's Classes'. This year there were only five pupils, two in March and three in July. 'This is, I am convinced, a useful extension of the work of the Laboratory', writes Allen in his Report to the Council; most zoologists of to-day would agree that the carrying on of these classes by Garstang, Orton, and their successors has been as important a piece of work as any that the Association has done. In the following year fifteen students came at Easter and three in the summer; now, for over 40 years, only war has interrupted the sequence of classes: not only have they been full to overflowing but so have been the similar classes which have followed them in other marine laboratories. Several thousand biologists must already have benefited from this new departure made by Garstang and Allen in Allen's first year.

Bethe, working at the crustacean nervous system, obviously came to Plymouth to be in the same laboratory as his fellow-worker, Allen, with whom he became great friends, as he did with me. I should like to record that with my own microscope, in my own well-examined preparation, when I requested him to recognize, looking down on a collar-cell, the stained cross-section of a flagellum $0{\cdot}5\mu$ in diameter, he said, 'Oh yes, stained on its surface, with a white lumen.' Which I could then see, but had not seen.

Late in August E. T. Browne came for a six weeks' visit, lodging over me at 7 Hoe Park Terrace. Browne's company was always very welcome to Allen; they had been at University College together, and I think, to the end, Browne was his most intimate friend and always his staunch ally. He was a learned, industrious and skilful zoologist, with a passion for his medusae. Like Riches, he suffered from the apparent infinity of time before him in which to publish a *magnum opus*, but he loved zoology, and the prosperity of the Laboratory became one of his greatest interests. He was gentle and much liked.

I had to spend month-ends in London, and on my return on August 31 Allen greeted me with startling news. There was to be a congress of journalists in Plymouth for the third week of September, they might be 400; the local Committee had approached Allen as to facilities for seeing the Aquarium and he had invited the congress to a soirée.

For a moment my breath stopped as I pictured our poverty-stricken, understaffed Association throwing a party to four hundred strangers—then I saw Allen's genius! He invited them to the most imposing building of the neighbourhood outside the Citadel; in the Aquarium, the great laboratory and adjuncts, there was comfortable walking and standing room for two hundred people, there was that for them to look at which they could not see elsewhere in Britain and nothing in laboratory or aquarium to show our penury. It was a type of all that he did as Director—to see what advantages we had and to use them.

Then he fired his second barrel: 'The difficulty is that since Cunningham went to Grimsby in February, we have no fish expert here. Stead doesn't arrive until the 10th, and it wouldn't be fair to ask a man fresh from his tripos to take it on when he has only been a week in the laboratory. You will have to be our fish expert.' 'But I know next to nothing about fishes!' 'That doesn't matter, you've got nearly three weeks to learn them up in.' So, with microscope under its bell-jar, for the first half of September I read diligently in Couch and Day and the volumes of our *Journal*, or wandered round the Aquarium trying to memorize which fish was which.

The Soirée was a complete success—except for the fish expert! I don't think there were much short of two hundred guests, and it all went with a bang. There was a rocker-microtome on which someone cut sections. Browne had a beautiful show of hydroids and medusae, Riches had an appalling nemertine (was it 20 ft. long? or only 10?), there were half a dozen other shows, and the tanks lit up in the Aquarium and two or three printed leaflets to take away (I was put on to write one on making sections in paraffin), and every one was hard at it seeing things and all seemed to enjoy themselves. I gave lectures at the tanks to twenty or thirty young journalists, who listened eagerly to the carefully prepared stories. Unfortunately, they were joined by dear old Robert Bayly, one of our Governors and benefactors, who knew a good deal about fishes and was anxious to make the most of the opportunity of contact in the Aquarium with the Fish Expert of the Marine Biological Association. When his questions came—though I had not finished my stories—there was nothing for it but to look at my watch, say 'Excuse me, I'm wanted upstairs', and dash through the Director's House into the safety of the great laboratory.

The out-of-pocket expenses were about five pounds. Allen pleased many scores of people, he put the Laboratory on the map with at least a hundred journalists, he gave dignity to the M.B.A. before the face of Plymouth, and he added valuably to our self-respect.

Beaumont now became for many years the occupier of a table for the greater part of the year. He was a real naturalist, but led more to admire and enjoy than to investigate, and his greatest interest was yachting. At one period I laughed to Allen that I thought Beaumont only used his cubicle as a place in which to eat his lunch. But Allen was quite right to welcome him. He was very kindly and always ready to help anyone; he took his part in tedious identification of dredged samples and in considering problems concerning our boats. Principally, however, I think Allen saw the first urgency of building up a community to be a soul to the body of our buildings. Beaumont was an addition to that community, so was Hodgson. The Council allowed £100 for an 'Assistant to the Director', which in those days would have got him a good clerk. Instead he engaged, on a half-time basis, Hodgson, who had a job under the town council, as lecturer and later as Museum curator; having recently returned from the Antarctic expedition, on which he had been doing pycnogons and amphipods, he was naturally one of us.

The most important newcomer this year to the Laboratory, after the Director, has not yet been named. He was an old acquaintance of mine, A. J. Smith, who was assistant to Brockett in the Morphological Laboratory at Cambridge. Adam Sedgwick said in the winter: 'I don't know what to do about Smith. He is too good a man to go on in his present position and I can't offer him anything better. Do you think he would be of use at Plymouth?' I presume that Sedgwick put the same question to Allen, for Smith began in April that career of 45 years through which he incurred the gratitude of every biologist working at Plymouth, and was always the most loyal supporter of the Director. That invaluable addition to our strength we owe to Sedgwick and Allen.

On July 30 Allen made, off Prawle Point, the first four hauls of over a hundred, made with dredges, trawls, etc., during this and the next two years, to determine the relation between fauna and bottom deposits near the 30-fathom line from Eddystone Grounds to Start. He was enabled to do this by a Royal Society grant of £100 for boat-hire, and the Royal Society must have found his fine Report in 1899 (occupying No. 4 of Vol. v of our *Journal*) very ample justification for their assignment of this sum. Nearly everyone working in the

Laboratory assisted in the determination of the animals brought up, and Allen's friend R. H. Worth, the engineer and Devon archaeologist and geologist, gave essential help in analysis of the bottom deposits. It was a valuable effort in fundamental research, and the characteristic of the man was the skill with which our meagre resources were marshalled to undertake, and to carry through, such an important piece of team work.

Before I left at Christmas, Allen gave me another job, not involving false colours this time. The Colonial Secretary this year was the Right Hon. Joseph Chamberlain, who had been our champion in obtaining for us from the Government, first £500 a year and then £1000 a year. (He told me, when I showed him round the Stazione at Naples, that he would have liked to have got more for us, 'but you know those fellows Harcourt and the others take no interest in such things'.) It was quite natural, therefore, that he should instruct the Colonial Office to request from the Director of the M.B.A. a 'Report on Sponge Culture and Projects for the Improvement of Sponge Fisheries'. Allen asked me to look up the thing for him and show him the papers on which to found his Report. I did so, and wrote a note of my own (of which the Appendix may be the best part), and he made a business-like Report on the literature. I have just been re-reading some of his Reports in our *Journal* of these early years: 'Reproduction of the Lobster', 'Protection of Crabs and Lobsters', 'Fauna and Bottom-deposits'—and thinking what powerful writing there is in them. He does not deal in clichés, what he says he means; where there is a problem he puts to consider it the whole strength of a remarkable mind and writes his own opinion, clearly, logically, and briefly.

Early in this, his first year, the new Director made a revolution of far-reaching importance, with the object—to which he held tenaciously for 45 years—of enabling workers to rear delicate organisms in the Laboratory. The revolution was no other than a one-man revolt against the Crystal Palace tradition.

I can remember as a child the intense surprise and admiration elicited by the Crystal Palace Aquarium, where Londoners could see, living and moving, 'all' the denizens of the sea. The Crystal Palace was inland, 50 miles from the sea; Lloyd deserves the greatest credit for having designed a successful system whereby the tanks and reservoirs had only to be filled once, and then a few barrels to repair wastage sufficed to keep the exhibition brilliant. But when, shortly afterwards, he made the Brighton Aquarium, he used closely the same plan, which Westminster Aquarium of course followed. More remarkably, when Anton Dohrn founded his great Stazione Zoologica, he applied to Lloyd for advice, and Lloyd insisted that the closed circulation and the dark resting reservoir were essential to success. Lloyd had succeeded, so Dohrn followed his plan exactly, and nearly a quarter of a century after the opening of the Crystal Palace Aquarium, Lankester followed it at Plymouth.

But (*a*) the Crystal Palace had to have a closed circulation; Brighton, Naples, and Plymouth could take fresh sea water at negligible expense: (*b*) the closed circulation with the dark reservoir succeeded admirably for keeping in life the crabs, sea anemones, and so on, which I remember admiring at the Crystal Palace, or *Limulus*, a dozen of which they used to show us in the palmy days of the Brighton Aquarium; but you could not rear delicate larvae to be adults in that closed circulation. Allen threw overboard the Lloyd tradition, proclaimed that the less any tank is supplied with used water the better for its inhabitants, supplied the laboratory entirely with water which had never been under its roof before (passing it on to improve the aquarium circulation) and organisms lived in the laboratory which had hitherto been transitory visitors while soles spawned in the aquarium for the first time.

Already under the last directorship an occasional carboy of outside sea water was used in the laboratory, now those who requested it for delicate work had a wooden bucket of sea water brought to them from outside the breakwater, and in 1899 there was a pair of wooden 500 gallon reservoirs built at the top of the little stairs, from which by gravity 'outside water' was led through glass tubes to a few small jets in the laboratory; the cistern being replenished from a tank boat by force-pump.

As years pass the difference between the waters of Plymouth Sound and of the ocean becomes more and more noticeable, while the researches of the marine biologist become more and more delicate. Allen's plan of keeping the first reservoir supplied with high-tide water whenever it ran low had to be modified: it was found that often the Sound water was more poisonous than old Aquarium water, eventually new supplies were only taken rarely and the Aquarium acidity was corrected (I think Pantin devised this) with lime in the reservoir. Outside water in bell-jars took up the part it now plays, but never since Allen's revolution was the visiting naturalist advised to rear his larvae in many-year-old water drawn from Lloyd's resting reservoir.

Only by describing thus in detail the first year of Allen's directorship is it possible to give an idea of the character of the man and the reasons for his great success. One of his gifts it may not have brought out is his great business ability. With all his scientific abilities and knowledge and his passionate scientific interest he was a first-class business man.

He recognized at once that the first need to make the Laboratory live was to have it inhabited, and patiently, continuously, he warred against the desolation of barren tables. But his first year of work showed him another essential necessity: we must have a steamboat of our own. Hired boats, which the Royal Society had enabled him to employ, were too expensive to be used for ordinary collecting; also, even when most suitable, they had less than half the efficiency possible with our own boat, fitted with our own gadgets and our own special stores and a working cabin or deckhouse with tables, shelves, and appliances as experience proved necessary.

In January 1896 he reports to Council that 'the want at Plymouth which must be put before all others, is that of a suitable boat', 'the effective work of the Association could be increased by at least 50% if a suitable small steamer were in our possession'. On February, 13 1896 he adds, on the proof, a postscript to his Report: 'an opportunity of securing a small steam fishing yacht, upon advantageous terms, has presented itself...the Council decided it would be advisable to purchase without delay. The yacht, the *Busy Bee*, of Fowey, is now in our possession.'

She was 60 ft. long, 9 ft. 8 in. beam, gross tonnage 22·5—a giant compared with the gallant little *Firefly*, 38 ft. long, which we bought in 1889 and used hard for three years, or her successor *Pansy* of about the same size, given a bad name and sold again. That the prompt purchase of *Busy Bee* was wise is fairly proved by the fact that she served us well for five years, increasing our effective work nearer 100% than 50%. Allen paid £605 for her, spent about £85 in giving her a steam winch and other fittings for our work, and sold her in 1901 for £700.

In 1897 she replaced the hired sailing trawler with which sample periodic trawls had been made in the (nominally) closed bays since October 1895. She was a movable centre of work on such occasions, spending a fortnight each at Exmouth, Dartmouth and Falmouth. She worked hard, well and cheaply, but by 1901 Allen began to feel her too small. Our researches extended farther and farther into and up and down the Channel, for safety we needed something more powerful and for accommodation something larger; for each reason something beamier.

Keeping a watch on the lists of yachts for sale, Allen at length saw an offer which seemed very likely. The *Oithona* was a schooner steam yacht, 84 ft. long, 16 ft. beam, gross tonnage 69. Writing from memory, she had been built in 1876, but her engines were younger, she had been in the hands of a wealthy owner and—which attracted us most—her last cruise was all round Great Britain. A yacht 25 years old is not everyone's meat: she had been on the hard at Southampton all a year, and her price was now very moderate.

I think Allen and Beaumont went over to Southampton to look at her and that then Allen had her surveyed. Everything in her was of the very best quality, but her boiler was near its last legs. Allen bought her, with some small expenses, for £858. 8*s.* making £12. 10*s.* a ton as against £27 a ton for *Busy Bee* with a sound boiler.

There were of course alterations to be paid for, including the essential steam winch and deckhouse laboratory. When they were finished and she got to work, Allen was met by the verdict, 'That she is not a good sea boat and cannot be trusted to go far in doubtful weather.' For a vessel which has carried an expensive family all round Britain this seemed a strange conclusion. If it was final we had thrown away our chance—but the circumnavigation was a definite fact. We arranged to make our own judgement and took her out on a short trip when there was a good sailing breeze from the south-west.

There was no doubt, she was a wet ship! We met only little waves, but every one we went into came down on her deck. In a rough sea, clearly she would not only be uncomfortable, she would be alarming. We considered the matter carefully, there on board. Since her voyage from Lands End to John o' Groats and back we had added above deck level a heavy winch and a deck-house. These must be compensated, and to cure her dipping her nose we must lighten the bows.

Her chain cable was coiled absolutely in her eyes, resting on the inner surface of the cutwater. We ordered that to be coiled a few feet farther aft, behind a low bulkhead. There were two rather stouter chains as bowsprit shrouds, we condemned them. Then Allen became relentless—the bowsprit must go and be replaced by a light pole! It was a *beautiful* spar, and its great length finished her graceful proportions, but he was quite right: with that extreme leverage its great weight was a big load for her fine bows. So we condemned it, and one felt the trim rectified. There was still the question of buoyancy, and we now decided that her mainmast must be replaced by a pole (with the deck-house she could no longer ship a proper mainsail) and then Allen suddenly condemned the handsome teak panels which lined the bulwarks. It seemed a great pity—but teak is a heavy wood and 200 × 3 ft. of it a weighty deck ornament. I forget the weight that we estimated we were removing, but when we next went on the *Oithona* her deck remained dry, and she presently took on as her job a triangular voyage with its base over the Continental Shelf.

In her fourth year Allen replaced her long-suffering boiler and gave her a refit costing £847, so that with the winch, etc. she stood us in £1864. She was worth every penny of it. We worked her for some twenty years and sold her after the War to a Belgian biological station for £775. She was then due for extensive repairs—boiler-casing gone and boiler not too grand—and with the more extensive voyages we were undertaking (to trace the supplies of water to the Channel) we wanted something that was built to rough it: Allen did not like sending her to rough weather out of soundings. He felt very strongly his responsibility for the lives of those on our ships; he used to arrange for the *Huxley* to take the winter quarterly voyage when we were still doing the International Investigations.

We parted from the *Oithona* with great regret, she was a kindly ship.

The *Huxley* was bought by Allen and me at Grimsby, but I made Allen speak the deciding word as to the price. When she was sold, the profits on the whole transaction founded the 'Ray Lankester Investigatorship'. *Salpa* was wished on us by the Government and her price fixed. The *Huxley*, *Oithona* and *Busy Bee* were each thoroughly profitable as monetary investments, their purchase having been settled by Dr Allen, the specialist on the nervous system of embryonic crustacea.

I lived at Plymouth for four years (1898–1902), so our friendship became very intimate, the more as I was elected to the Council in 1899, so that we collaborated at the Council table as well as in the Laboratory. In 1898 Browne's plungers were in use—the first had been set in motion on September 4

1897. At this time Allen and Browne were still experimenting on arranging an inverted funnel as a plunger so that it emits at its lowest position a continuous stream of air-bubbles (now known to be a work of superfluity). The plunger has made a revolution in all aquatic biological laboratory research and made easy what used to be impossible. But few who have not read Browne's paper (*J. Mar. Biol. Ass.* vol. v, p. 180) appreciate Allen's share in the invention: Browne says, 'It was his suggestions which led to the plunger being worked by such a simple method.' Browne's part was the discovery that the essential to healthy aquatic life is the movement of the water, Allen's part was the siphoned jar for a motor, which put the plunger at the disposal of the humblest laboratory that owns a tap.

Garstang returned in June 1897 as Naturalist, starting in that capacity a successful partnership of work with Allen as Director which lasted until, ten years later, Garstang was appointed Professor of Zoology in Leeds University. His lively and original mind helped to develop the fishery side of our investigations, particularly as to the passage of sea water into and up the Channel, to test which he instituted considerable bottle experiments and treated the results with great ingenuity. The following five years saw much good work done at the Laboratory with increasing efficiency and smoothness; but the bitter shortness of funds still continued. With Bles succeeding Calderwood as Director, the receipts for 1892–3, deducting donations and composition fees, were £1932. 16*s.* 6*d.* In 1897–8, the fourth year of Allen's directorship, the receipts after deducting donations and composition fees were £1973. 13*s.* 2*d.* and the donations and composition fees deducted were only £65. 9*s.* whereas in 1892–3 they were £226. 5*s.* 6*d.*: so that Allen had the spending of £160 less than Calderwood and Bles. Allen had done splendidly; there were twice the number of visiting naturalists, our capable little *Busy Bee* was working hard, our scanty salaries fund was stretched to cover a small staff, competent although disgracefully ill-paid, all kinds of people were working hard. The *Journal* shows a very fine output of valuable work, both from staff and visitors. Yet to keep up that great building, with engine, pumps, Laboratory and Aquarium, to keep our fishing yacht collecting, to pay all wages ashore and afloat, the salaries of the Director and Naturalist and anything that we could squeeze out from time to time for additional help in investigation, we received still under £2000; say, the family expenses of two average professional men in Kensington.

In 1899 Allen was getting very much discouraged. It was not only that he had to save candle-ends and starve his staff, it was the absence of prospect of anything better. And indeed five years later the Plymouth income was actually no more, though the gross receipts were £100 higher because of increased sale of specimens and of fishing gear, which involved fully the £100 in extra expenses. Allen talked to me seriously of throwing it up and earning his money in something that was not heart-breaking—perhaps business? The only post to which I could have helped him would have been a business one at about

Dr E. J. Allen and Prof. E. W. MacBride
British Association, Leeds, 1927

the salary he was earning but not taking a third of the amount of work, so that he would have had more time for researches. To my great relief—as a member of the M.B.A.—this did not tempt him, and he put his neck into the yoke again.

Then in 1902 came the dawn! The Government agreed to take part in the International Exploration of the North Sea, and invited the M.B.A. to undertake England's share in the work for three years, offering us £18,500 for the whole job. The one saving clause was that in the 6 months to March 31, 1903 we were to receive £7200, leaving £11,300 (really £5500 a year) to March 31, 1905. This enabled us to pay the rent for the *Huxley*, of £870 a year, with £2000 down and £305 a year in the second and third years, easing the latter years and knocking some 40% off the cash to be found for purchase of the *Huxley*; hence the result already recounted. The International Investigation money was of course for the staffing and maintenance of the research vessel and a shore laboratory at the harbour where she was based. Little money came Plymouth way; but Allen was able to give to some of his seamen, as well as to some of his naturalists, a lift up into the Lowestoft staff; a little of the work was done by Plymouth, and in any case we were all part and parcel of the great International Exploration, and no longer a voice crying in the wilderness.

Yet, when it was all over, and Lowestoft handed to the Board of Agriculture and Fisheries, and the *Huxley* sold, and Garstang teaching at Leeds—we were a little better off, but not much. The *Huxley* was sold in 1910 and the Development Commission gave us £500 a year in consideration of our doing some work for the International Investigation which could be better done by us than by the Agriculture and Fishery Board at Lowestoft. The Worshipful Company of Fishmongers now gave us £600 a year. Allen improved the tank room and receipts increased, more tables were rented, the prices of our specimen trade were increased and it more nearly paid its expenses. In 1914 our total income was close on £3500; the Director's salary was raised to the princely sum of £300, but what pleased him much more was that instead of only a Naturalist working under him he now had a Hydrographer, Senior Naturalist, Second Naturalist, Additional Naturalist and Assistant Naturalist. There was nothing grand about the scale of the Laboratory of the Marine Biological Association of the United Kingdom, nor fitting to the dignity of the United Kingdom; but the 70% addition to our gross income made it a place where one had not the piteous sense of wasted work and opportunity which was so oppressive before 1903: it was modest, and everyone was underpaid, but it was efficient.

Then, after the War, when the other biological stations sent S.O.S. appeals to the Government to save them from extinction, we started building.

Sir W. B. Hardy guided with genius the reorganization of biological effort in Britain to receive the benefits of the Development Fund through the

Ministry of Agriculture and Fisheries. It was for us the transformation scene of the pantomine. Allen's long labour was at length not in vain, the last third of his Directorship was passed at the head of the leading Marine Biological Station of the world, adequately maintained. The balance-sheet for 1926–7 shows as salaries: 'Director £1062. 10*s*., Physiologist £910, Naturalists £3211. 15*s*. 4*d*., Hydrographer £538. 6*s*. 8*d*.', making in themselves a total of three times the whole expenditure of 1895.

It was a great happiness to him to be the much loved head of this fine machine. The last time I went round with him as Director there were eighty biologists working in the Laboratory, yet we could both remember its echoing to our footsteps in 1894. To have wrought that change was a life's work of which a man might well feel proud.

I have not written of his research. It is not for me to appraise it; that has been done by every reading zoologist and especially by the Councils of the Royal Society and the Linnean Society. I think if he had been working as the Fellow of a College, instead of Director of the Laboratory, he would probably have kept on at the crustacean nerves and other histological work that suggested itself: he had the delight which only the histologist knows in looking with high magnification at the form and structure of cells. Exigencies of his organized survey turned him to polychaetes, and he then became extremely interested in the mysteries of regeneration. The water question—how to keep delicate organisms alive in a laboratory, he regarded as challenging every biologist with its urgency and its difficulty. He had taken up the glove and waged the battle for fifteen years. There again, in dutifully solving the problem obstructing our research, he discovered that no artificial sea water would grow more than a tiny tuft of diatoms, but that if 10 c.c. of real sea water were added to the litre and the whole sterilized—then the diatoms filled the jar. Hopkins had just published his 'vitamines' and the comparison interested them both extremely.

I think that in science, as in administration and in business, his leading talent was the rare one of discerning the essential.

But a picture of Allen's intellectual equipment is not given by describing alone the excellence of his research and the insight of his philosophical writing (Presidential Address, British Association, Section D, 1922; Hooker Lecture 1929, *Proc. Linn. Soc.*). Though it never made him dissipate his energies, he had the delightful gift of interest in everything. He said to me once in the 'nineties that people talked a great deal of rubbish about the oppressive mass of zoological literature, that it was perfectly possible to read it all as it came out, more or less carefully according to the paper, and that, so far as the available libraries went, he did it. Thirty years later he said, 'It is now completely impossible'; but the width of his biological reading was equalled by only a few men whom I have known. What you only found out by degrees was the extent to which he kept himself up to date in physiology, chemistry and physics; what you might never find out was the amount of his general reading and his keen love of poetry.

Given this mind and this learning, with the invention that devises new methods and new lines of research, adding to it his unlimited sympathy and great unselfishness, it is possible to understand how it was that few of those who worked at Plymouth ever failed to receive valuable help from the Director. His unswervingly honest mind and sensitive kindness made him the ideal critic. In the second and third decades of this century he became really important in the zoological world; I thought at one time that in him we were going to see again someone who could be called the leader of contemporary English zoology—but Lankester was the last who could be given that title and will remain the last. There was, however, no question of the widespread respect and affection shown to Allen wherever zoologists met together, and well-deserved distinctions began now to come fast. F.R.S. in 1914 was followed by the presidencies of the Devonshire Association in 1916 and of Section D of the British Association in 1922; a year later Denmark gave him the Hansen Memorial Medal and Prize, three years after that the Linnean Society awarded Allen their Gold Medal and in 1929 he gave their Hooker Lecture. Edinburgh conferred on him the Hon. LL.D.; he was made Foreign Member of the Royal Academy, Denmark; in 1935 he received his C.B.E. followed in 1936 by the Darwin Medal of the Royal Society and the Agassiz Medal for Oceanography from the National Academy of Sciences, Washington. University College, London, elected him a Fellow in 1939.

An end comes to everything, and in 1935 he told the Council that he wished to resign. He was sixty-nine and he felt the work too heavy for him. At the Council's request he agreed to stay on to September of the following year, but six weeks before the appointed terminus he was taken seriously ill and had to have an operation. While he was laid aside, his colleagues effected the transfer of his possessions to rooms already chosen in Reservoir House, Skardon Place, belonging to Mrs Sexton, his connexion by marriage as well as a valued colleague and collaborator; so when he left the nursing hostel he moved into his new home. It is a house of the Regency time, with the grace of proportion belonging to its period: the bow windows of his sitting-room looked over the garden to the reservoir; over the roofs beyond the water he saw Staddon Heights and the Sound. The attractive old garden, made pretty by Mrs Sexton's discerning care, is bounded by a low wall which forms the top of the reservoir retaining wall; the place offered a pleasant harbour for last moorings.

Here he set to work to cultivate wood-lice, and found them extremely interesting—as he would have found cockroaches or frogs or mustard and cress. But after two or three years he had to give up research because he found his memory had gone too much (the biographers tell the same of Faraday). When I was last at Plymouth, two years ago, he was hard at work reading modern physics, particularly the text-book just published in joint authorship by his brother and his sister's son, Roland Maxwell. 'I forget it all entirely in three months, but that does not spoil the interest.'

He was quietly happy, walking over to the Laboratory several times a week, and in contact with many old friends; but I think he found his rooms a little solitary after having lived a third of a century in the focus of a busy hive. He enjoyed his very occasional sallies up to London to stay with relations and friends and go to a meeting or two. He was up in April 1942 and spent 24th–29th at Cambridge, happy and making others happy. In November he had some bronchial trouble and was poorly the first days of December. By December 4th he seemed to have thrown it all off. He died suddenly and painlessly on the morning of December 7.

Allen was the best-loved of British zoologists, of whom most hailed him as a personal friend. With a shy manner, he was always unobtrusive and rather silent, always helpful, always transparently honest intellectually and morally, and always kind. He had a delicate reverence for women which they completely appreciated and he was delightful with children, who were all ready to adopt him as a new uncle or grandfather. With all his friendships, however, family affection held the central place with him. Very early in his directorship he brightened the Director's House with a long stay from his fifteen-year-old sister. I often heard of his brothers: it is unusual for two brothers each to be a Fellow of the Royal Society (Professor H. S. Allen, F.R.S., of St Andrews, is his younger brother), but he told me that their eldest brother Dr H. N. Allen (Principal of the Poonah College of Science), who died in 1932, was abler than either of them. When he received his own F.R.S. in 1914 and I congratulated him, he said bitterly, 'Why didn't it come when I should have valued it, it is too late now'—the value of such things being to show one's parents. That was temporary, his well-earned honours were not lost on him.

I knew Allen for half a century and I never knew him do a mean thing or a selfish thing. He was understandingly sympathetic and unfailingly helpful. Of all his gifts, his greatest excellency was as a friend.

G. P. B.

(*The plate facing page* 680 *is reproduced by courtesy of The Yorkshire Evening Post.*)

STUDIES IN THE FUNCTIONAL MORPHOLOGY AND EMBRYOLOGY OF *ONCHIDELLA CELTICA* (FORBES AND HANLEY) AND THEIR BEARING ON ITS RELATIONSHIPS

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(Text-figs. 1–6)

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INTRODUCTION

Onchidella celtica, the only British representative of the family Onchidiidae, lives between tide marks in crevices of eroded rocks on the north and south Cornish coasts. Along the exposed southern coast between Rame Head and Looe many of the rocks rise to a steep escarpment on the north side, and here in the sloping crevices, sheltered from wave action and from strong sunlight, the animals are found in considerable numbers. They occur a few feet down from high-water mark, not above it as mentioned by Ellis (1926), and some are found in rocks uncovered only at low tide. They live in communities ranging from two or three individuals to over sixty, and in each community animals of varying ages are found. On warm days, and when uncovered by the ebbing tide, the molluscs emerge from their shelter in search of food, but during the colder months, between November and March, they remain in the rock crevices; then they are very inactive and pass through a period of hibernation. A similar annual period of limited activity is described by Arey & Crozier (1921) for *Onchidium verruculatum*.

When wandering over the rocks *Onchidella celtica* avoids long exposure to strong sunlight and the wind-swept surfaces; in stormy weather it does not appear. Not every animal emerges at an appropriate tide, though the majority are active on still summer days when the sky is overcast, or there is a heavy sea mist, and then they may browse on the southern slopes of the rocks which are not visited in sunny weather. Their food consists of young algae and encrusting diatoms, and in search of these they travel with considerable rapidity: the sensory lobes of the head always in contact with the substrate, the tentacles protruding from under the anterior margin of the mantle, and the posterior edge of the mantle upturned to expose the pulmonary aperture, which is then widely open. They may wander a yard or so from the rock crevice from which they emerged, selecting food at intervals and eating it ravenously, or their journey may only be a few inches; they frequently visit the beds of young mussels or barnacles to rasp the organisms which settle on the shells. At the rising tide the molluscs retreat to their shelter and this they reach before the water covers them. Although some exceptions have been observed, on the whole each animal returns to its own particular crevice, or one near by; the exact path of the outward journey is not retraced. This homing habit has been previously observed in *Onchidium floridanum* (Arey & Crozier, 1921), which lives on the shore rocks at Bermuda. The crevice which is chosen may have an extremely small opening to the exterior, is always sandy, and mingled with the sand are diatoms washed in by the waves; these the animals often eat. Forbes & Hanley (1853), in citing observations made by Mr Couch, state that *Onchidella celtica* occurs between Polperro and Fowey about a foot or two from the sea, and migrates up and down the shore as the tide rises and falls to maintain its distance from the water. No such migration has ever been seen and the animals appear to live at the same level throughout the year. During the summer months the egg capsules are laid in the rock crevices inhabited by the adults, and within the capsules the embryos develop to a creeping stage which superficially resembles the parent.

The anatomy and development of *O. celtica* have been described by Joyeux-Laffuie (1882). This is the only member of the Onchidiidae in which the development has been studied. No sections were made of the embryos and this probably accounts for his misinterpretation of some points in their structure, and his failure to elucidate from the development certain features in the anatomy of the adult. A little histology of the alimentary and reproductive systems of the adult is given, but the correlation between the structure and function of the various organs is only slight. Although Joyeux-Laffuie mistook the lung of *O. celtica* for a kidney which had acquired some respiratory function, yet he recognized the mollusc as a pulmonate which in some features approached the nudibranchs; with this Brock (1883) agreed. Since Cuvier (1804) described the anatomy of *Onchidium Peronii* and pointed out its close resemblance to the slugs, most investigators have accepted the Onchidiidae as pulmonates, though their exact affinities within this subclass have been subject to controversy; in a number of characters they stand isolated from the air-breathing molluscs. Bergh (1885) states that they are derived from land snails, and represent a terminal twig in the evolution of the Stylommatophora. On the contrary, Plate (1893), who studied the anatomy of several members of the family, concluded that their origin must be sought in the tectibranch opisthobranchs, and hence he considers that they are more closely allied to the Basommatophora. Von Wissel (1898) maintains that they approach this order more closely, though he is unable to decide whether they are primitive or degenerate. In a comparatively recent paper Labbé (1934) describes the occurrence of siliceous spicules in the tissues of several members of the Onchidiidae, and he claims strong opisthobranch affinities for the group, consequently he renames it the Silicodermatae and places it under the opisthobranchs. The pulmonates he groups with the prosobranchs and gives the following classification of the gastropods:

Prosobranchs: Prosobranchs *sensu stricto*;
Pulmonates.
Opisthobranchs: Opisthobranchs *sensu stricto*;
Silicodermatae.

The work here recorded was done at the Marine Laboratory, Plymouth, and at Birkbeck and Royal Holloway Colleges. I wish to express my indebtedness to the University of London for the use of their table at the Plymouth Laboratory, and to Birkbeck College for a grant towards the cost of publication.

The Alimentary Canal

The head lies between the anterior edge of the mantle (Fig. 1A, *am*), which covers it dorsally, and the foot (*f*), and is separated from the latter by a deep groove which leads to the opening of the pedal mucous gland (*o*). The mouth (*m*) is ventral, and when the animal is not feeding it appears as a longitudinal slit, bordered on each side by a muscular and sensory lobe (*sl*) which has a broad, flat, ventral surface. Dorsal to each lobe is a tentacle (*t*), black in colour as the rest of the head and bearing an eye (*e*) at its apex. When the animal is resting in the rock, or if it is disturbed during its perambulations, the tentacles are invaginated and completely withdrawn, and the whole head region may be protected by the anterior border of the foot covering it ventrally. The mouth leads into a short oral tube which is a ventral prolongation of the buccal cavity and can be closed by a sphincter. The sensory lobes and the oral tube are covered by a cuticularized, columnar epithelium, through which penetrate the ducts of subepithelial glands. Some of these are mucous cells producing secretion for the lubrication of the surfaces; others are plentiful on the sensory lobes and contain small colourless spherules—not dissolved in any fixative, staining black with iron haematoxylin and probably secreting a cement. On the anterior wall of the oral tube, below the point at which it opens into the buccal cavity, the cuticle is thickened to form a transverse ridge with a sharp and slightly irregular summit. The ridge acts as a jaw against which the radula can work, and assists in biting the food which is drawn in by the radula. A mid-dorsal ciliated channel runs down the length of the buccal cavity to the oesophagus, and is separated from the more ventral part of the cavity by a longitudinal fold of connective tissue on each side. The ventral part is covered by cuticularized columnar cells, and its floor is hidden by the odontophore which projects forwards to the oral tube. A large number of mucous cells lie in the connective tissue beneath the epithelium and open between the columnar cells. Ventral to the anterior tip of each longitudinal fold is the opening of a salivary duct which passes back and penetrates the buccal musculature; when free from this it swells into a small vesicle which is surrounded by a thick coat of circular muscles, and then runs down the side of the buccal mass for a short distance to be encircled by the white flocculent lobes of the gland. This spreads ventrally to the nerve ring, to which it is attached by connective tissue. The gland is made up of mucous cells, and scattered rather infrequently among these are secreting cells of a different type: their protoplasm is vacuolated and the vacuoles contain small spherules which stain deeply with iron haematoxylin and orange or red with azan. The cells, all with large nuclei and well-marked nucleoli, are grouped in clusters, and from each of these a short duct opens into the main salivary duct, which is also

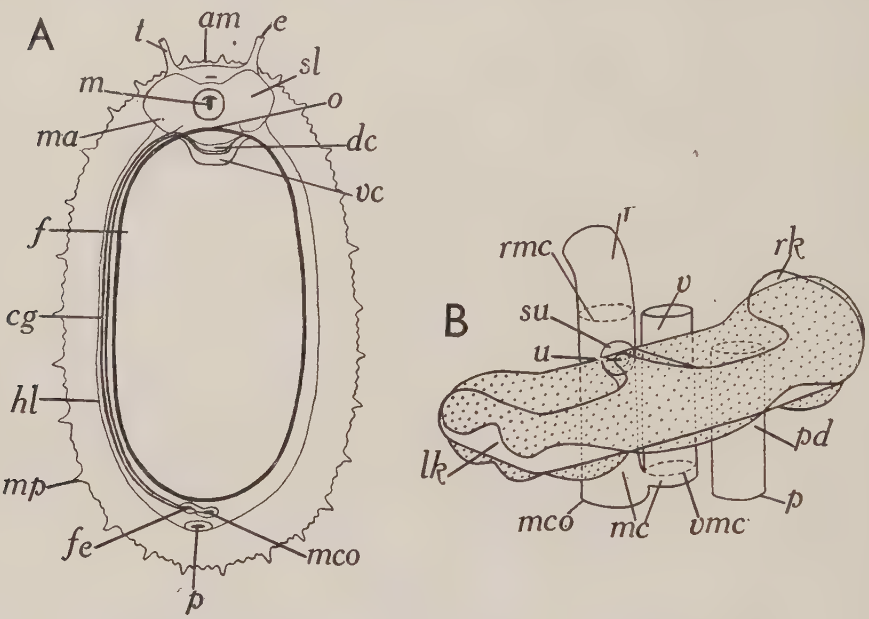

Fig. 1. A. Diagrammatic ventral view of *Onchidella celtica* showing the position of the external apertures and the course of the ciliated groove: ×4. B. Diagram showing the opening of the rectum, secondary ureter and vagina into the mantle cavity, and the position of the pulmonary duct. The stippled area represents the lung wrapped around the kidney: × about 8. *am*, anterior edge of mantle; *cg*, ciliated groove; *dc*, dorsal chamber of pedal mucous gland; *e*, eye; *f*, foot; *fe*, female aperture; *hl*, hyponotal line; *lk*, left limb of kidney; *m*, mouth; *ma*, male opening situated beneath sensory lobe; *mc*, mantle cavity; *mco*, opening of mantle cavity previously called anus; *mp*, marginal papilla; *o*, opening of pedal mucous gland; *p*, pneumostome; *pd*, pulmonary duct; *r*, rectum; *rk*, right limb of kidney; *rmc*, opening of rectum into mantle cavity; *sl*, sensory lobe; *su*, opening of secondary ureter; *t*, tentacle; *u*, opening of primary ureter; *v*, vagina; *vc*, ventral chamber of pedal mucous gland; *vmc*, opening of vagina into mantle cavity.

surrounded throughout this part of its course by gland cells. The ducts are ciliated and between the epithelial cells open the surrounding gland cells. Their secretion is passed along the ducts by the cilia, and pumped into the buccal cavity by the muscular vesicle. In the connective tissue which binds the gland cells together are a few muscle fibres.

The two dorso-lateral longitudinal folds of the buccal cavity extend towards the middle line and fuse with one another at their free edges at the beginning of the oesophagus, so that the ciliated dorsal channel runs imperceptibly into the oesophagus. This is directed back over the buccal mass and curves ventrally to pass through the nerve ring. Behind the nerve ring it forms a capacious crop which describes a curve to the left before leading back to the stomach. The whole oesophagus is characterized by a well-developed musculature. The lining is thrown into pliable longitudinal folds (Fig. 2, *lf*) by the variation in thickness of the underlying connective tissue; when the crop is full the folds are completely obliterated. Joyeux-Laffuie (1882) states that they increase the absorbing area, but no absorption takes place in this part of the gut. The epithelium is composed of columnar cells, which are ciliated in the anterior oesophagus as far as a region just behind the nerve ring and in the posterior part of the tube which opens into the stomach; between these two areas the epithelium is cuticularized. The nuclei lie towards the base of the cells, and above them the cytoplasm contains small yellow pigment granules. A large number of mucous cells are distributed in and below the epithelium, most numerous in the anterior oesophagus. Circular and longitudinal muscles run through the connective tissue and radial muscles penetrate the folds; the circular muscles form a sphincter around the entrance to the buccal cavity and the stomach.

The structure of the stomach may best be studied when it is filled with food (Fig. 2), for when empty the thin-walled regions are contracted and their full extent is not realized. The most conspicuous feature is the large globular triturating sac or gizzard with its massive dorsal (*d*) and ventral (*v*) plates, which are hinged to one another laterally. The oesophagus (*oe*) does not open directly into this sac, but communicates with its anterior end through the thin-walled initial chamber (*ic*) of the stomach. This receives anteriorly and dorsally the right (*rd*) and left (*ld*) ducts from the corresponding anterior lobes of the digestive gland. The intestine likewise is separated from the gizzard by a distensible pouch, the terminal chamber of the stomach (*tc*), and this overlies the initial chamber. The terminal chamber opens ventrally into the initial chamber and posteriorly into the gizzard. From each of the two anterior digestive gland ducts emerges a fold, the two folds running back parallel with one another along the dorsal wall of the initial chamber and then curving dorsally into the terminal chamber, along the ventral wall of which they pass forwards. These longitudinal folds, separated only by a deep gulley, terminate in a small caecum (*c*) which occupies a mid-ventral position at the origin of the intestine (*i*). The left one is larger than the right and curves around the left and posterior margins of the opening of the caecum before entering it; the right fold runs straight into the pouch. The excretory matter from the anterior ducts of the digestive gland is directed along the folds and so by-passes the triturating stomach. Similar longitudinal folds are described and figured by Plate (1893) for *Onchidium verruculatum*, and their occurrence in such diverse groups of the gastropods as chitons (Fretter, 1937), *Patella* (Graham, 1932) and tectibranchs (Fretter, 1939) suggests that they constitute one of the characteristic features of the gastropod stomach. There is a third liver duct in *Onchidella* (*pd*) which arises from the distensible posterior wall (*pw*) of the gizzard, and passes ventrally to the smallest of the three lobes. The excretory matter from this less important duct has no directed course to the intestine. When the stomach is empty it can be seen that the longitudinal folds of the oesophagus (*lf*) continue down the ventral and lateral walls of the initial chamber. This is ciliated and rich in mucous cells; in histological detail and in musculature it resembles the posterior oesophagus. The histology and musculature of the terminal chamber is also similar, but the epithelium is more deeply folded. The folds (*ft*) arise at the opening into the triturating stomach and pass to the region of the caecum, where they end abruptly. Epithelial mucous cells are frequent, increasing in number towards the caecum, in which they are sufficiently numerous to alternate with the ciliated columnar cells. Their cytoplasm, which is vacuolated and contains small colourless secretion spherules, stains with iron haematoxylin; it is little affected by mucicarmine, though the discharged secretion, which usually fills the pouch, is susceptible to this stain. The nuclei of the secreting cells are round, basal and nucleolated. The caecum is muscular and the muscle fibres are mainly circular in direction. The dorsal gizzard plate (*d*) lies anterior to the ventral one (*v*). Their crushing surfaces are covered by a smooth cuticle, and the

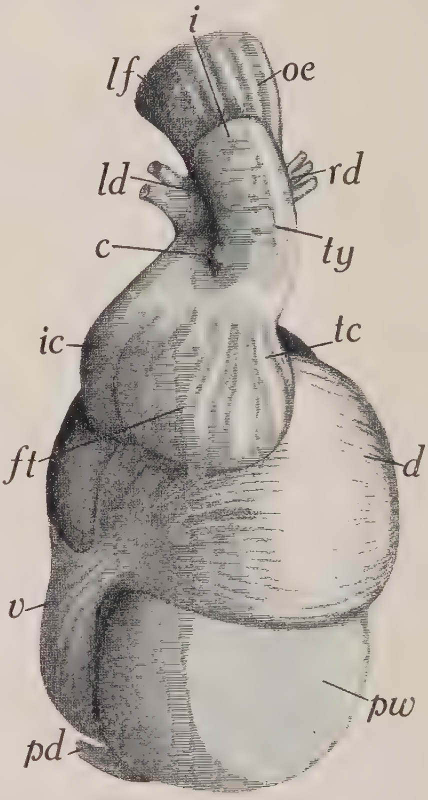

Fig. 2. Distended stomach seen from above and slightly to the left: × 17. *c*, caecum; *d*, dorsal plate; *ft*, longitudinal fold of terminal chamber; *i*, intestine; *ic*, initial chamber; *ld*, left duct of digestive gland; *lf*, longitudinal fold of oesophagus; *oe*, oesophagus; *pd*, posterior duct of digestive gland; *pw*, distensible posterior wall; *rd*, right duct of digestive gland; *tc*, terminal chamber; *ty*, typhlosole; *v*, ventral plate.

subepithelial tissue comprises alternating bands of circular and longitudinal muscles. Laterally the muscle layers are thin and are covered externally by compact connective tissue which binds the two plates together. Elsewhere the walls of the gizzard are lined by columnar ciliated cells, and are thrown into deep longitudinal folds when the stomach is empty; a thick layer of circular and longitudinal muscles surrounds the epithelium and assists the triturating action. In the epithelium which secretes the thick cuticle of the plates the cytoplasm is vacuolated, and immediately above the basal nuclei it contains, in each cell, a small clump of darkly staining spherules; distally a few pigment granules may be seen—these are most distinct in young animals in which the cuticle is thin. Fibrillae run through the cytoplasm and merge into the most recently secreted cuticle, which stains lightly with iron haematoxylin and blue with azan. The older part has a different staining reaction and is black after iron haematoxylin and red with azan.

The digestive gland lies dorsal to the reproductive organs and for the most part anterior to them. Of the three lobes the posterior one is by far the smallest; it embraces the posterior wall of the triturating stomach and spreads beneath it. The right and left anterior lobes extend anteriorly as far as the buccal mass, covering on their respective sides the oesophagus and stomach, and the right mass forms a packing for the coils of the intestine. Blood vessels, opaque white in colour since they are coated in tissue rich in crystals of some calcium salt, branch over the surface of the gland. The epithelium is composed of three types of cells arranged according to a definite plan. The most frequent type is the digestive cell, which lines the tubules except in the crypts at the angles of the wall, and here the two other types, the excretory and lime cells, occur exclusively. The digestive cell arises from a narrow base to a height which exceeds that of the other cells. The distal border is regular and slightly rounded in starved animals, but in animals which have recently fed it has an irregular outline. The cytoplasm is vacuolated throughout and the oval nucleus, with a very definite nucleolus, lies towards the base. The vacuoles are smallest at the free ends of the cells, where the cytoplasm is more dense, and they increase in size proximally. The tiny vacuoles contain colourless secretory spherules, and the deeper ones are filled with brown or green particles which, immediately above and below the nucleus, may be aggregated into large clumps. Fat droplets are also found in vacuoles in the more distal cytoplasm. In the crypts, which are constricted off from the rest of the tubule so as to project into the haemocoel, the excretory cells are frequently clustered around the lime cells. They are club-shaped with a rounded free end and are taller than the lime cells which are triangular in longitudinal section with a broad base. The cytoplasm of the excretory cells is vacuolated and the vacuoles contain brown homogeneous spherules of excretory matter which are not dissolved in any fixative. They vary in size: there may be a large number of tiny brown spherules in one cell, or fewer spherules of a larger dimension, or all may be fused into one dark brown clump. The nucleus lies beneath the excretory masses, and is readily distinguished from the nucleus of the lime cell which is larger and has a more prominent nucleolus. In the lime cells the cytoplasm is dense and very vacuolated, and the vacuoles are filled with lime spherules.

From the terminal chamber of the stomach the intestine passes over the surface of the digestive gland, and just beneath the buccal mass, to the right side of the body cavity. It dips ventrally under the anterior aorta and then curves dorsally again to describe an anterior U-shaped loop. From this it leads back, above the digestive gland and the reproductive organs, to a mid-ventral opening behind the posterior edge of the foot. The intestine possesses throughout its length a dorsal typhlosole (Fig. 2, *ty*) which arises opposite the ventral caecum of the stomach. The epithelium is thrown into folds which, together with the typhlosole, are formed by variations in the thickness of the underlying connective tissue. In the anterior part of the intestine, and up to the point at which it passes beneath the aorta, folds arise on either side of the typhlosole, and each runs obliquely backwards around the lateral wall to terminate mid-ventrally. In the posterior part of the tube the folds are directed longitudinally and, except in the rectum, are less pronounced. The columnar ciliated epithelium is taller over the typhlosole than elsewhere and is rich in gland cells. These are of two kinds—mucous cells and club-shaped cells which are filled with small spherules and have a more limited distribution: few are found at the anterior end of the intestine, they increase in number towards the dorsal loop, in which they are numerous, and beyond this are entirely lacking. The spherules stain black with iron haematoxylin and red with azan: when secreted they swell to form a white viscid fluid which binds together the faecal matter. Such cells are not found in the epithelium of the typhlosole. Mucous cells occur in all parts of the epithelium; they are most abundant in the anterior intestine and some lie in the connective tissue beneath the typhlosole. The cytoplasm of the ciliated cells contains small pigment granules which impart to the wall its yellowish brown colour. A double muscular coat consisting of inner circular and outer longitudinal layers surrounds the epithelium, and radial muscles run through the connective tissue of the folds.

When the animal starts off on its feeding foray the crop and stomach are empty of food, containing only a brownish fluid, with a few sand grains in the triturating stomach; when it returns both are fully distended and the contents consist mainly of pieces of algae—filaments up to a centimetre or more in length—and innumerable diatoms. Mixed with these there may be scraps of sponges, foraminiferans such as *Polystomella* and *Gromia*, and a mass of sand and detritus. The food is rasped from the rock surface and drawn into the dorsal part of the buccal cavity by the radula, while the sensory lobes take a firm grip of the feeding ground to anchor the head in position. The radula works against the chitinous jaw and is lubricated by a copious secretion of saliva. The viscid salivary fluid also binds together the food particles. The cilia on the dorsal channel and the anterior oesophagus help to direct the food into the crop, although here, as in other parts of the gut, its passage is mainly effected by muscular action. It is passed into the stomach as more food is taken in, and both regions of the gut act as a storage place; the contents are digested at leisure during the full tide.

The lumina of the tubules of the digestive gland rarely contain any particulate matter: a few diatom cases may be found there, but these are not taken up by the cells of the gland—in fact no particulate matter appears to be ingested. In animals which have fed on a mixture of diatoms, algal filaments and insoluble iron saccharate the iron has never been traced into the digestive cells, though large quantities are present in the gut and a few particles in the liver tubules. If soluble iron is eaten, it is absorbed by the digestive cells and appears in a very weak solution in the terminal vacuoles. Later it passes to the deeper parts of the cytoplasm and is adsorbed to the green and brown masses which fill the vacuoles—these are apparently accumulations of excretory matter, and at intervals they are expelled from the cells and passed to the intestine. Such observations suggest that only the soluble products of the food are ingested by the digestive gland, and that particulate matter is only carried into the ducts accidentally. Throughout the gut there is no other site of absorption. The ingesting cells have also a secretory function and the free tips of the cells, which contain small spherules, are cut off from the epithelium and pass into the stomach and crop.

An examination of the faeces shows that a large part of the food which is eaten is indigestible since no cellulase appears to be present, and some is undigested. Except for mechanical action the cell walls of the algal filaments are unaffected by their passage through the gut; it is only the contents of cells which have been opened that are utilized. The diatoms constitute a large proportion of the animal's diet and the majority of these are digested, though a number appear in the faeces still with their protoplasmic contents.

The food is crushed by the mechanical action of the stomach; when this is full all the chambers exert pressure on the contents forcing them towards the triturating sac. In this way they are compressed by the dorsal and ventral plates. At the same time the food is mixed with the enzymatic fluid secreted by the digestive gland, so that the available digestible parts are broken down. The soluble products of digestion are wrung from the food and forced into the liver ducts—the two anterior ones receiving the greatest quantity. The fluid is prevented from being regurgitated into the oesophagus by closure of the oesophageal sphincter. Whilst active the gizzard tends to rotate on its longitudinal axis, the dorsal and ventral plates swinging through less than 90° in a clockwise direction and then back to their original position. Their thick chitinous covering protects the walls against the large number of sand grains which are taken in with the food, and which also help in grinding it. The gizzard is rarely free from sand grains, and Joyeux-Laffuie (1882) considers them to be of such consequence that he likens them to the stones in the gizzard of a bird. Heidermanns (1924), in his study of freshwater pulmonates, suggests that the muscular stomach is developed in proportion to the quantity of sand eaten, and attributes to it the function of forcing the indigestible matter along the intestine. This it certainly does in *Onchidella*: when matter is to be passed from the stomach the gizzard drives its own contents forward, and since at the same time the initial chamber of the stomach contracts, the only available outlet is dorsal, into the terminal chamber, and thence to the intestine. If an animal be collected on its return from a meal and dissected about four hours later, food remains are massed in the intestine, although the crop and stomach are still amply distended. Frequently the animal takes in more food than the digestive system can cope with, and the quantity of undigested material in faeces which are discharged within a few hours after a meal is greater than that contained in later faecal masses. The pellets are rod-shaped and may be a centimetre or more in length. The sand grains, detritus, algal filaments and diatom cases, of which each is composed, are compacted and covered by a cementing fluid. Firmly attached to each pellet is a longitudinal beaded strand of excretory matter from the digestive gland, and directly opposite this is a shallow longitudinal groove—the impression of the typhlosole. The excreta from the digestive gland are moulded into characteristic form by the caecum, in which the longitudinal folds from the anterior liver ducts terminate. The flow of waste matter passing along the folds feeds the caecum, and when this is full its contents are moulded by its muscles, and cemented, by the viscid fluid from its walls, into a compact oval mass which is then expelled. A succession of pellets is thus formed connected end to end, and plastered against the flow of faecal matter from the stomach, to which it firmly adheres. This function of consolidating and moulding the faecal matter from the digestive gland is also attributed to the caecum of *Aplysia* (Howells, 1942). The small quantity of waste from the posterior liver duct is voided with the food remains from the stomach. The excretory phase of the digestive gland, which follows one of absorption, involves the digestive and excretory cells. If animals are injected with soluble iron saccharate, the iron is taken up by the three different types of cells in the gland, though most active absorption occurs in the crypts, where it either forms small spherules in the young excretory cells or is added to the brown spherules already present in the older ones. In the lime cells it appears as minute masses in the dense cytoplasm between the calcium spherules. Its ultimate fate has not been traced. Small quantities of iron are taken up by the digestive cells and accumulate with the food remains in the basal vacuoles; with these they are expelled from the distal ends of the cells and passed from the gland in the faecal stream. At the same time the oldest excretory cells, which are filled with brown spherules or in which the spherules have fused into one large clump, leave the epithelium. It would appear that the function of these cells is to take up waste matter from the blood and build it up into concentrated masses which accumulate until the cell becomes effete. A similar function has been suggested for cells in the digestive gland epithelium of tectibranchs (Fretter, 1939).

The passage through the intestine is lubricated by mucous secretion from the epithelium. The muscles of the tube compress the faecal matter and fashion it into a smooth rod, and the fluid from the club-shaped gland cells binds the particles together. Since the excretory matter from the digestive gland is directly opposite the impression made by the typhlosole, it is attractive to imagine that this structure exerts a pressure which helps the excretory matter to adhere to the faecal rod. During defaecation variable lengths of the rod are voided; the greater length of the intestine may be emptied at one time.

RESPIRATION

One of the most interesting features of the Onchidiidae is the presence of a lung which opens by a small pneumostome (Fig. 1A, *p*) at the posterior end of the body, slightly to the right of the mid-line and behind the openings of the anus and vagina (*fe*). Owing to an inadequate knowledge of its anatomy and histology the lung has been the subject of much controversy in times past: Joyeux-Laffuie (1882) and von Jhering (1877) maintain that it is a kidney which has to some extent taken over a respiratory function, whilst Semper (1876–7) and Bergh (1885), on the contrary, state that it is primarily a respiratory organ and quite distinct from the kidney, although they maintain that the two open into one another. According to Plate (1893) it is a reduced mantle cavity which has no connexion whatsoever with the kidney, since the latter discharges through the ureter into the rectum. Von Wissel (1898) accepts this interpretation, though Haller (1894) opposes it.

When an *Onchidella* moves actively over the rocks which are uncovered by the tide, the posterior tip of the mantle is raised to expose the pulmonary aperture. This is widely open and may remain so for some minutes. The opening leads through a short duct to a spacious cavity which, in sections, can be traced forwards on either side of the median body cavity as branching diverticula; these extend to the level of the posterior end of the heart. The lung is thus U-shaped and it follows the course of the kidney with which it is closely associated. The kidney, however, extends farther forwards than the lung: its right limb spreads around the median wall of the pericardium, and under the ventral wall, as far as the anterior tip of the heart. The reno-pericardial duct opens into the posterior end of the pericardial cavity. The left limb is less developed than this, but passes forward to about the same level. The U-shaped part of the haemocoel, in which lie the kidney and (on the right side) the heart, is separated from the general body cavity by a muscular diaphragm. This is incomplete anteriorly on the right side, so that the lateral and median haemocoelic spaces communicate by a wide opening, and through this passes the anterior aorta. Branching diverticula of the lung penetrate between the lobes of the kidney and push into the tissues of the mantle, so that they come into intimate contact with the blood spaces.

The lips of the pneumostome are lined by a columnar ciliated epithelium which is surrounded by subepithelial mucous cells; the cilia beat out of the lung cavity and guard against the entry of small particles. A section through the delicate respiratory tissue of the lung is shown in Fig. 3A. A thin outer sheet of squamous cells (*os*) lines the cavity, and a corresponding inner layer (*is*), which rests upon the tissues of the mantle (*tm*) or the muscles surrounding the kidney tubules (*m*), runs parallel with it, the two being separated in most places by a series of channels formed by cells of similar histological character running at right angles to the two layers (*sc*). Blood from the surrounding haemocoelic spaces (*h*) runs through the network of channels, and is thus separated from the lung cavity only by the rather structureless and lightly staining outer sheet of squamous epithelium. Here and there a mucous cell pours its secretion on to the respiratory surface. Should the mollusc be immersed in water the pulmonary opening closes immediately, and, since the cavity decreases in size with the contraction of the muscles of the mantle, air escapes through it. The diaphragm and muscles

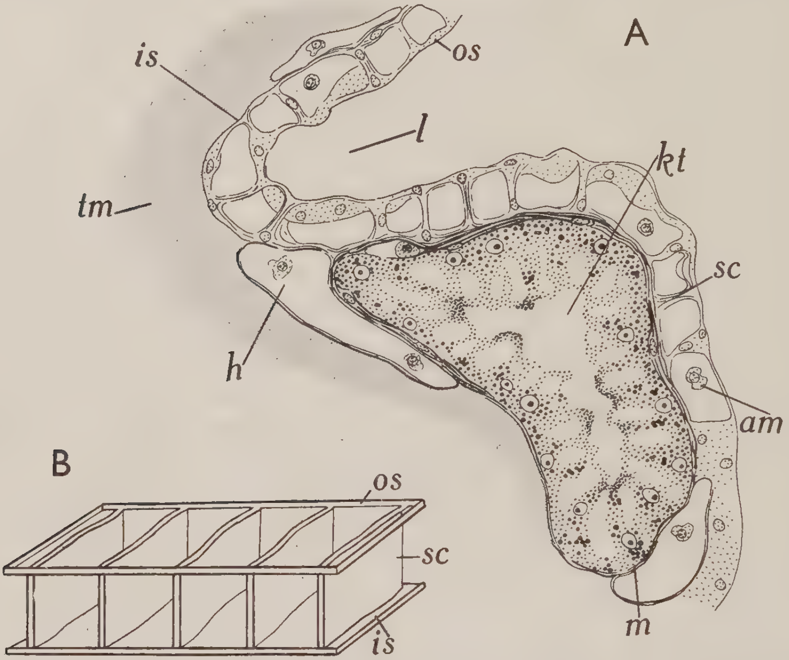

Fig. 3. A. Transverse section through wall of lung, underlying kidney tubule, mantle and associated blood spaces: ×400. B. Diagram to show arrangement of blood channels in wall of lung: ×about 580. *am*, amoebocyte in blood channel; *h*, haemocoelic space; *is*, inner sheet of squamous epithelium; *kt*, lumen of kidney tubule; *l*, lumen of lung; *m*, muscles surrounding kidney tubule; *os*, outer sheet of squamous epithelium; *sc*, squamous cells subdividing space between *is* and *os*; *tm*, tissues of mantle.

surrounding the kidney tubules may also be concerned with the ordinary respiratory movements. Normally the animal has settled in its crevice by the time of the rising tide, and the aperture has closed and remains so until the next period of activity. In the sluggish interlude pallial respiration is adequate.

The surface of the mantle is papillated and when exposed to the air is moistened by the secretion of pallial glands: these are unicellular and are grouped in clusters between the cuticularized epithelial cells. If the mollusc be removed from water and the dorsal surface thoroughly dried, it is covered, within a few minutes, by fluid from the glands. Along each side of the mantle project a number of marginal papillae (Fig. 1 A, *mp*) which are larger than the rest and equidistantly placed. Each bears at its apex a small pore through which, when the animal is irritated, a spirally coiled stream of white secretion is discharged. The secretion is produced by a large flask-shaped multicellular gland, which is embedded in the thickness of the mantle at the base of the papilla (Fig. 6C, *mg*); it is squeezed from the various types of cells sharing in its composition, and forced through the long straight duct, by a surrounding muscle layer of considerable thickness. The histology of the glands appears to be similar to that of *Oncidiella* (= *Onchidella*) *marginata* and *O. juan-fernandeziana* (von Wissel, 1898). The marginal or peripheral glands in *Onchidium* are referred to by Arey & Crozier (1921) as repugnatorial glands, and it is stated that sea anemones and crabs refuse to eat food tainted with the secretion.

In *O. verruculatum* (Hirasaka, 1922) the dorsal papillae of the anterior two-thirds of the mantle bear eyes, and those of the posterior third are produced into tree-like gills. Neither of these are developed in *Onchidella*, though the respiratory importance of the mantle is very considerable owing to the interlacing network of blood capillaries below the epithelium. The mollusc can live under water for long periods, relying entirely on pallial respiration; its activities, however, are then reduced. It can also rely on this alone when it is inactive at low tide. Pulmonary respiration supplements pallial respiration when the respiratory requirements of the animal are increased during activity out of water, but experiments show that either is sufficient by itself to keep the animal alive for at least a week. If the dorsal surface be vaselined and the animal placed in a humid atmosphere, the pulmonary aperture is opened and the lung is used continuously; frequently the edges of the mantle are upturned as if in an attempt to increase the respiratory area. The behaviour appears to be more normal when the pneumostome is blocked with vaseline, and specimens thus treated may occasionally wander about, though only over a limited distance. Should both mantle and lung be thrown out of action death follows in a few hours.

Although *Onchidella* is on the whole restricted to damp situations in which the humidity conditions approach those of saturation, the fact that in mid-August it can be seen to leave the shady rock and travel over sunny surfaces, shows that it can survive, at least for a short time, in lower humidities. In order to investigate the degree of desiccation which the animal can resist specimens were subjected to relative humidities of less than 100%. The required humidities were obtained by placing dishes with sulphuric acid-water mixtures of known specific gravity in the bottom of large jam jars, and placing above the open dishes a platform of perforated zinc. Eight animals were introduced into each jar and a glass plate was sealed to the opening with vaseline. The animals appeared to show a distinct negative geotaxy: they invariably attached themselves to the glass plate or to the sides of the jar, and rarely descended as far as the zinc platform. Edney paper hygrometers were used for measuring the humidity. The results are contained in the following table:

Temperature 12–13° C.

75% R.H.	Little activity, lung rarely used. All dead or moribund after 2 days.
80% R.H.	After 2 days all appeared shrivelled and inert. 7 dead within $3\frac{1}{2}$ days.
90% R.H.	No apparent effect of dryness for 3 days.
95% R.H.	Lived for 7 days before showing signs of desiccation Quite active, pulmonary aperture frequently open.
100% R.H.	All appeared normal and active.

When introduced into a humidity gradient ranging from 75 to 95% R.H. the animals made no attempt to aggregate in regions of optimum humidity—they are completely akinetic.

The Reproductive System

The account of the reproductive system given by Joyeux-Laffuie (1882) includes little histology, and important points in the anatomy are overlooked. Plate's (1893) and von Wissel's (1898) descriptions of other members of the Onchidiidae indicate that throughout the family the gross structure of the reproductive organs is remarkably uniform, though in no case is the detailed course of the male and female ducts elucidated, and such essential structures as the fertilization chamber, with its associated receptaculum seminis, and the large prostate gland on the male duct have not previously been described.

In *Onchidella celtica* the yellowish brown hermaphrodite gland, which lies in the posterior part of the body cavity, is divided into two main lobes, each acinous in structure. Young individuals are protandrous, but later both types of sex cells are found in all acini of the gland, with the spermatozoa always far more numerous than the ova. The ducts through which the genital products pass to the hermaphrodite duct are ciliated. The proximal part of the hermaphrodite duct acts as a vesicula seminalis; it is coiled in a close spiral which passes forwards for a short distance on the left side of the haemocoel. The epithelium is columnar and ciliated, save for a longitudinal strip of gland cells, which, in animals dissected during late summer and autumn, appears as a deep brown streak; in spring there is little or no external indication of the glandular area. The pigmentation is due to brown refringent granules in the highly vacuolated protoplasm of the gland cells. The free ends of the cells may be irregular in outline and here the protoplasm is more dense than elsewhere. Sperm are entrapped by the dense protoplasm and engulfed; they have never been seen in the deeper layers of the cells, so the granules which accumulate there are probably products of sperm digestion. A similar activity on the part of the corresponding region of the male duct has been described by Linke (1933) in three species of *Littorina*, and also in *Ocenebra erinacea*, *Nucella lapillus*, *Nassarius reticulatus* and *Buccinum undatum* (Fretter, 1941). The distal part of the hermaphrodite duct is closed off from the vesicula seminalis by a sphincter, and turns abruptly to the right to lead straight back towards the hermaphrodite gland. At the end of this part of its course it winds into a knotted mass of fine coils, which are not visible superficially, since all are bound together by connective tissue and covered externally by a connective tissue sheet supplied with circular muscles (Fig. 4, *chd*). The fine coils lead anteriorly and open into a wider region of the duct (*hd*) which runs forwards and ventrally, through the flocculent mass of the albumen gland, to a small fertilization chamber (*fc*). The opening into this wider region lies at the base of a shallow longitudinal groove which leads posteriorly to the opening of a small caecum (*c*), and continues along the duct anteriorly for a short distance. The caecum has a narrow neck and broadens at its blind end; the epithelium, which is covered by extremely long cilia, is thrown into a few deep folds, and these subdivide the lumen. It is referred to as the vesicula seminalis by Joyeux-Laffuie (1882), though he failed to realize that the proximal part of the male duct acted as such, and actually only on one occasion have a few sperm been found in the pouch. Its size appears to vary throughout the Onchidiidae: in the genus *Onchidella* it would appear to be generally small since von Wissel (1898) describes it as a small diverticulum, about 0·5 mm. in length, in *Oncidiella* (= *Onchidella*) *marginata* and *O. juan-fernandeziana* and alleges that it is absent in *O. coquimbensis*. In the genus *Onchidium*, however, it is considerably larger and is figured by Plate (1893) in *O. verruculatum* and *O. nigrum* of such a size that the name vesicula seminalis is well merited. Hence it would appear that in the genus *Onchidella* it is an incipient or vestigial structure. The distal part of the hermaphrodite duct is lined throughout by a ciliated epithelium, with circular muscles beneath the basement membrane and longitudinal fibres outside. The cilia are long in the coiled region (*chd*), but in the caecum they may exceed five times the height of the cells; here, also, the muscle coat is very thick.

The ducts of subepithelial glands open between the ciliated cells which line the fertilization chamber (*fc*). The glands are embedded in a layer of connective tissue, and their cytoplasm contains small secretion spherules which stain deeply with iron haematoxylin. A few may lie in the epithelium. The chamber receives paired ducts, one from the left and the other from the

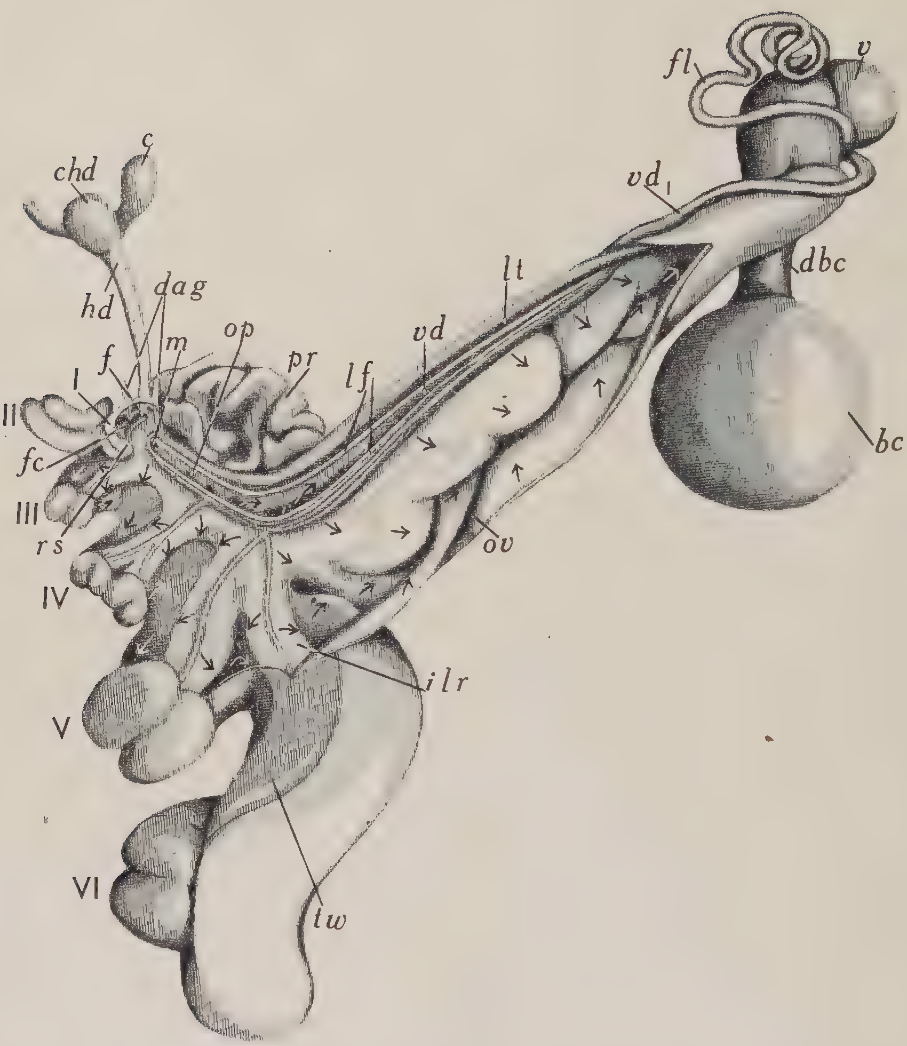

Fig. 4. The reproductive ducts: ×20. The middle glandular section of the hermaphrodite duct has been slit open longitudinally and the duct uncoiled. The albumen gland has been removed. *bc*, bursa copulatrix; *c*, caecum; *chd*, fine coils of hermaphrodite duct covered by connective tissue; *dag*, duct of albumen gland; *dbc*, duct of bursa copulatrix; *f*, opening of hermaphrodite duct into oviduct; *fc*, fertilization chamber; *fl*, flagellum; *hd*, hermaphrodite duct; *ilr*, internal longitudinal ridge of oviducal pouch; *lf*, longitudinal flange; *lt*, longitudinal tract leading from receptaculum seminis to duct of bursa; *m*, opening of hermaphrodite duct into vas deferens; *ov*, oviduct; *op*, opening of prostate; *pr*, prostate; *rs*, receptaculum seminis; *tw*, thin wall of spiral caecum; *v*, vagina; *vd*, vas deferens; vd_1, non-glandular part of vas deferens; I–VI, oviducal pouches.

right lobe of the albumen gland (*dag*). This is orange in colour and the two lobes are bound together by connective tissue and lie dorsally over the oviduct and vas deferens. Their ducts are short and ciliated, and each is formed by the union of smaller collecting ducts. The secreting cells, all of a single type, are grouped in small clusters which are bound to one another by a thin layer of connective tissue. The cytoplasm is filled with yellow, granular spherules and these stain an intense blue with azan, are not affected by mucicarmine, and stain rather deeply with iron haematoxylin.

The lumen of the hermaphrodite duct, on approaching the fertilization chamber, appears as a longitudinal slit, which becomes divided into left and right channels by a slight constriction. The duct runs beneath the chamber for a short distance and the left channel, which is the larger of the two, opens laterally into it (*f*); the opening is opposite those of the albumen ducts. Through this passage the eggs are conducted to the oviduct (*ov*). The right channel, on the other hand, is the beginning of the vas deferens (*m*), but, although here the male and female passages are separated from one another, during the next part of their course the separation is merely functional. The vas deferens (*vd*) runs back, accompanied by the female part of the duct (*ov*), towards the posterior end of the body, lying to the right of the hermaphrodite duct. It is a deep gulley, V-shaped in transverse section and surrounded by a thick layer of subepithelial glands. During the proximal part of its course the apex of the V is directed ventrally, and the oviduct, a similar longitudinal glandular groove, lies on its left, the two passages having a common dorsal wall. A longitudinal ciliated flange (*lf*) on each side of the mouth of that gulley which acts as vas deferens, closes it off from the oviduct. On the dorsal wall of the female channel, above the flanges guarding the male section, is a longitudinal ciliated tract (*lt*) leading upwards to the receptaculum seminis (*rs*) and downwards to the duct of the bursa copulatrix (*dbc*). Near the origin of the male duct lies the opening (*op*) of the large prostate gland (*pr*). This is a sac-like outgrowth of the glandular walls of the vas deferens, and its walls are deeply folded. The gland cells here and in the vas deferens are grouped in clusters, bound together by connective tissue, and their long ducts open between ciliated cells which direct the secretion along the male duct. The gland cells have large spherical nuclei with very prominent nucleoli and the secretion spherules which fill the vacuolated cytoplasm stain with iron haematoxylin; those in the ducts take the stain most readily. After azan the spherules are red and the cytoplasm purple. In the vas deferens muscle fibres, circular and oblique in direction, pass through the connective tissue between the glands, and also form an external layer which is concentrated on the right side of the gulley and around the dorsal wall.

The common genital duct, with its parallel male and female divisions, passes back through the haemocoel and becomes twisted in such a way that the vas deferens appears to curve around the right side of the oviduct and then to pass across its dorsal surface to the left side. The twist causes the openings of the two channels to face not dorsally, as in the anterior region, but to the left. At this point the vas deferens becomes completely separated from the oviduct, and for the rest of its course it is a narrow, ciliated and non-glandular duct with muscular walls (vd_1). It passes beneath the female duct to the origin of the external ciliated groove (Fig. 1 A, *cg*). This leads from the female aperture (*fe*), and passes around the right side of the body near the junction of the foot and body wall, to the pedal mucous gland. The vas deferens enters the muscles above the groove and runs parallel with it. Just behind the male opening (*ma*), which is situated a short distance behind the right tentacle, it returns to the haemocoel and runs back in a sinuous course to the base of the retracted penis. During the final part of its course mucous cells, which are wedged between the fibres of the thick coat of circular muscles, open into the narrow tube; the penis sheath is also lubricated by subepithelial mucous and mucoid glands. The retractor muscle of the penis is very long, and from the base of the copulatory organ it runs straight back in a ventral position to its origin on the dorsal surface of the foot beneath the bursa copulatrix.

The oviduct is complicated in its anterior part by the development of six pouches which are outgrowths of the glandular wall (Fig. 4, I–VI), and which serve to increase the distance through which the eggs must travel on their way to the female aperture. The first of these pouches is very small; it overhangs the fertilization chamber and acts as a receptaculum seminis (*rs*). The walls at the blind end are an opaque white, and resemble in histological detail the glandular walls of the fertilization chamber. In all other parts the glandular area of the oviduct is yellow. The remaining five pouches are of increasing size, with the sixth very much broader and longer than the others—it is coiled in a spiral and may exceed the whole of the rest of the oviduct in length. The four pouches anterior to it (nos. II–V) lie over the proximal end of the female channel. In other members of the Onchidiidae more than one spirally wound outgrowth from the genital duct has been described: two are figured by Plate (1893) in *Onchidium verruculatum* and three in *O. nigrum*, and these extra spirals appear to correspond with some of the smaller pouches in *Onchidella celtica*.

The wall of the oviduct is composed of columnar ciliated epithelium resting upon a thick and compact layer of gland cells which pour their secretion into the lumen of the duct. Along each pouch the epithelium is folded to give an internal longitudinal ridge (*ilr*). In the long spiral caecum (no. VI) that part of the wall directly opposite the ridge is thin with few subepithelial glands (*tw*). The base of the first pouch and the second, into which are directed the fertilized eggs with their coat of albumen, produce a mucous secretion. In the two succeeding pouches (nos. III and IV) the secretion is similar, but stains less easily with mucicarmine and slightly with iron haematoxylin. In the last two pouches (nos. V and VI), the largest of the series, the same two kinds of cell occur, and also a third type of gland which gradually replaces the other two and is especially numerous in the spiral pouch. The protoplasm is vacuolated and the majority of the spherules contained in the vacuoles are untouched by mucicarmine, stain rather deeply with iron haematoxylin and deep blue with azan. At certain centres of activity within each cell clumps of mucoid spherules occur, though they are not found in the ducts of the glands, and the final secretion is untouched by stains which are considered to be specific for mucus. The subepithelial cells of the main channel of the oviduct, which passes from the spiral caecum towards the female aperture, are mucoid glands, and in the connective tissue which binds them together in groups run a few circular and oblique muscle fibres. Such fibres enable the oviduct to be closed off from the rest of the genital duct. The gland cells decrease in number after the separation of the male and female channels, and the oviduct becomes a ciliated tube which is surrounded by a very thick coat of muscles. It curves ventrally and then describes a slight dorsal loop which receives on its dorsal wall the duct of the bursa copulatrix (*dbc*). The longitudinal ciliated tract (*lt*), which arises anteriorly near the receptaculum seminis and runs back between the original dorsal wall of the genital duct and the ciliated folds over the vas deferens, curves to the right with the twisting of the common genital duct, and, after the separation of the vas deferens, runs along the dorsal wall of the ventral curve of the oviduct to the opening of the duct from the bursa. The bursa (*bc*) lies anteriorly beneath the common genital duct; it is a large spherical vesicle and on dissection appears strawberry-pink in colour and flecked with a white calcareous deposit. It is lined by tall glandular cells with oval, nucleolated nuclei lying in the lower half of the cytoplasm. This is vacuolated throughout and the small secretion spherules contained in the vacuoles are dissolved on fixation. The free ends of the cells often have an irregular outline due to the discharging of the secretion. Beneath the basement membrane of the epithelium are a few circular muscles, and outside these the connective tissue contains blood spaces surrounded by large cells which are laden with calcium granules. The lumen of the bursa is invariably filled with a reddish brown fluid and in this may be found irregularly shaped refringent masses of a similar colour. During spring and summer there are clumps of spermatozoa, and a few living sperm have been seen there in December. The fluid is derived from the prostate secretion and from the wall of the vesicle, though neither of these secretions are so deeply pigmented when first liberated. The duct from the bursa (*dbc*) is lined by tall, columnar, ciliated cells and surrounded by a thick coat of circular muscles, with which are interspersed longitudinal fibres. It runs beneath the posterior end of the oviduct, and around its right side, to open at a point which marks the beginning of the vagina (*v*). The vagina, a very muscular tube, passes ventrally to the female aperture which lies to the right of the anus. A long flagellum (*fl*), with yellow glandular walls, coils over its dorsal surface and opens into it ventrally by a short duct. The opening is just posterior to the opening of the duct from the bursa. Joyeux-Laffuie (1882) refers to the flagellum as the caecal appendage or mucous gland, though the latter name is misleading since the secretion is not of a mucous nature. A fine canal runs through the flagellum and is lined by gland cells and ciliated cells; in the duct a columnar ciliated epithelium occurs and there are no gland cells. The secreting cells, which have basal nuclei, contain spherules of varying sizes clustered at the distal end of the cytoplasm. The ciliated cells are constricted by the gland cells and the nuclei are elongated and situated in the mid-region of the cells. A coat of circular muscles underlies the basement membrane and forms a sphincter around the duct. In the vagina the epithelium is columnar and ciliated, and the connective tissue below is ramified by radial, circular and longitudinal fibres, between which are mucous cells; externally a thick and compact coat of circular muscles forms a sphincter.

The vagina opens to the right and slightly in front of the so-called anus (Fig. 1A, *fe*, *mco*). A groove connects the two apertures and the lips of the groove run into those of the vaginal aperture, on the one hand, and those of the anus on the other. The ciliated groove (*cg*), arising at the female aperture, is lined by a columnar epithelium with short cilia. The epithelium is ridged longitudinally, both because of the fact that the supporting connective tissue varies in thickness and because the cells are of unequal height; the surface is lubricated by a few subepithelial mucous glands. A muscle coat of considerable thickness lies in the connective tissue, the fibres being mainly circular in direction, and the groove can be deepened so that the longitudinal ridges are obliterated, and the two lips then approximate to form a virtual tube. Although several attempts have been made to study the ciliary currents on the walls, only on one occasion was movement observed—this was very slight and directed anteriorly. The groove leads to the pedal mucous gland, which lies above the anterior tip of the foot and is a deep pouch with an extensive transverse opening (*o*). At its inner end the pouch is divided into two by a horizontal muscular partition, and the ciliated groove, which passes into the gland on the right side, runs straight back and ends in the shallower, dorsal chamber (*dc*). A cuticularized epithelium lines the walls of the undivided part of the gland, and the whole dorsal chamber except for the right wall on which the ciliated groove runs. The cuticle is pierced by the ducts of subepithelial gland cells. The dorsal chamber is very muscular, most of the muscles being circular, a few longitudinal or oblique. The ventral chamber (*vc*) is glandular, and the secreting cells, which are subepithelial, surround the ventral and lateral walls, opening to the surface between ciliated cells, whilst the dorsal wall is cuticularized. The secreting cells are for the most part mucous and mucoid glands, the former being especially numerous laterally. In the undivided part of the pedal gland the secreting cells decrease in number. In addition to mucous and mucoid cells two other types of secreting cells are present, and a few of these spread into the ventral chamber: in one, which is ubiquitous in the tissues of the foot, and is also found around the ciliated groove and the dorsal chamber of the pedal gland, the secretion is in the form of large protein spherules; in the other the cytoplasm is homogeneous and granular, but contains one or two large vacuoles with no apparent contents.

Egg masses of *Onchidella celtica* are found in the crevices inhabited by the adults and several may occur together. Each mass, which is laid by one individual and contains from sixty to over a hundred eggs, is somewhat flattened and of an irregular outline, measuring about a centimetre in diameter. It is firmly cemented to the rock, in most cases to the roof of the slanting crevice, and consists of the irregular coilings of a long tubular capsule which is embedded in jelly, and which is expanded at regular intervals by the eggs. Each egg lies at the centre of an oval mass of transparent albumen, and that in its turn is covered by the capsule wall. Between one egg and the next the capsule narrows to a fine strand. The capsule wall is made up of three distinct layers: an inner mucoid coat and middle and outer layers which, though thin, are very tough, the former staining lightly with iron haematoxylin and the latter deeply. The strand between any two eggs has a similar structure: in the centre is the mucoid layer, enclosing perhaps a streak of albumen, but the two outer layers are thicker than elsewhere since they are not distended, and here they appear to be made up of concentric sheets of a similar secretion which, in the middle layer, are separated by mucoid fluid.

Joyeux-Laffuie (1882) states that fertilization is reciprocal and Hoffmann (1929) figures the position of two copulating individuals in which the right borders of the mantles are in contact, the anterior ends pointing in opposite directions, and in each the penis is protruded from beneath the anterior edge of the mantle on the right side and thrust into the female aperture of the other. Sperm, embedded in prostatic secretion, are deposited in the bursa copulatrix, and in mature individuals collected during early summer they are present there in large numbers. Joyeux-Laffuie (1882) assumes that the eggs are both fertilized and encapsulated in the vagina, but a more detailed knowledge of the genital duct shows that this cannot be the case. From the bursa (Fig. 4, *bc*) the sperm make their way to the receptaculum seminis (*rs*) via the longitudinal groove (*lt*) which connects these two regions of the female tract, and there they await the discharge of the eggs into the fertilization chamber (*fc*). It is not known how soon after copulation the formation of egg capsules may begin.

When the eggs are passed down the hermaphrodite duct the proximal end of the vas deferens is contracted and they proceed along the wider female channel to the fertilization chamber. This is distended with secretion from the albumen gland and, one by one, the eggs are discharged into the secretion and are also fertilized. The stream of eggs and albumen now starts on the circuitous journey down the oviduct entering each of the six pouches (I–VI) in turn and being directed through these by the ciliary currents on their walls. Meanwhile the encasing capsule wall is secreted. The actual secretion of this wall has not been observed, though the part played by the various types of glands may be conjectured.

Prior to the entry of the fertilized eggs the glands of the oviduct discharge their secretion. In the first two diverticula of the duct this is mucous, and together with the mucoid secretion from the two succeeding diverticula it supplies the material for the manufacture of the inner layer of the capsule wall. The two outer layers are produced by the fifth and sixth diverticula, the largest of the series, with the sixth providing the most extensive glandular area of the whole oviduct. In these diverticula the mucous and mucoid cells are gradually replaced by glands which furnish a secretion with the same staining properties as the outer layer of the egg capsule; in no other area of the oviduct do such glands occur. The outer layer of the capsule wall is composed entirely of this secretion, whilst the middle one is composite, being an admixture of the inner and outer layers. In the posterior part of the oviduct the subepithelial glands lubricate the passage for the stream of eggs and add to it a thin mucoid covering. The exact locus at which the whole mass is acted upon so that it acquires its moniliform appearance is uncertain. It may be in the muscular vagina, or even outside the female duct if the process is purely physical.

The contents of the oviduct are presumably discharged into the ciliated groove. The cilia may at such a time become more active and combine with the muscles in effecting the passage of eggs along the groove and into the dorsal chamber of the pedal mucous gland. Since this chamber is very muscular the egg string may be moulded here. It seems reasonable to assume that the secretion in which they are embedded, and which cements them to the substrate, is poured from the glandular walls of the pedal gland, for this secretion has the same texture and staining properties as that supplied by the gland.

EMBRYOLOGY

The newly laid capsules of *Onchidella celtica* may be collected in July, August and the beginning of September; it is doubtful whether any are produced later. Within the capsule the embryo passes through a prosobranch veliger stage, followed by a post-veliger which shows gradual detorsion and a recession of the visceral hump, so that before hatching the appearance of the adult is assumed. In December and January empty capsules have been found in the rock crevices, as well as capsules containing veligers and post-veligers. The development of these was presumably retarded by the low temperature of the winter months, which may also account for the number of malformed individuals among them. Joyeux-Laffuie (1882) alleges that embryos kept in the laboratory, if not carefully attended, take on abnormal shapes, such abnormalities occurring more frequently in *Onchidella* than in other gastropods. It is the earliest post-veliger stages of detorsion which are most frequently malformed and among which there is the highest mortality. Spawn has been obtained from animals in captivity and from this normal embryos developed.

In such egg masses laid during the first week in July the veligers were fully formed in seventeen days, and about a month later their embryonic development was near completion—they appeared as miniatures of the adult. They hatched a week or so after. Only those points in the development which help to elucidate the adult structure are considered here, and the stages leading up to the veliger are completely omitted.

The Veliger

The veliger measures about 0·3 mm. in length, and by means of the large bilobed velum (Fig. 5A, v_1) it swims in the surrounding albumen. A pair of eyes (*e*) is developed, each situated on a slight protuberance which represents the rudiment of an adult tentacle. The foot (*f*) has a triangular sole and bears a large operculum (*op*) which was not observed by Joyeux-Laffuie (1882); near the broad base are embedded two otocysts (*o*), symmetrically placed, one on each side. At the anterior end of the foot the pedal mucous gland is present. The visceral hump is covered by a thin transparent shell (*sh*), which shows an endogastric coiling through about 180°, and on the right side is the mantle cavity (Fig. 6A, *mc*). This communicates with the exterior by a wide opening and at its extreme inner end is the anus. Projecting into the mantle cavity above the anus is a transitory organ, the larval kidney (Fig. 5A, *lk*), which corresponds in structure and position with what Mazzarelli (1906) calls the secondary kidney of the opisthobranchs; Joyeux-Laffuie (1882) mistakes it for the hermaphrodite gland. Sections show that it comprises three cells (Fig. 6A, *lk*), one much larger than the others. They are held together by a fine layer of connective tissue and their bases are directed upwards towards the dorsal surface. The nuclei are large and deeply staining, each with two nucleoli, and the cytoplasm is highly vacuolated; in the vacuoles small granules occur. It is uncertain whether this kidney communicates with the exterior: the suggestion of a minute opening has sometimes been seen, and it may be that the aperture is only visible when a drop of excretory matter is expelled. The rudiments of the definitive kidney of the mollusc can be found in a somewhat undifferentiated mass of cells (Figs. 5A and 6A, *rdk*) which lies above the larval kidney and between it and the pericardium.

Behind the velum and on either side of the oesophagus, in the young veliger, is a small opaque rounded body with green-brown granules, the so-called larval organ of Joyeux-Laffuie (1882). These are also transitory structures which cannot be traced in later stages, and appear to be homologous with the primary or cephalic kidneys of opisthobranchs and other pulmonates (Mazzarelli, 1906).

The mouth, which is median and situated beneath the velum, is surrounded by long cilia. It leads into the buccal cavity, a ventral diverticulum from which forms the rudiment of the odontophore. The oesophagus (Fig. 5A, *oe*) passes through the nerve ring (*g*), where the ganglia are concentrated as in

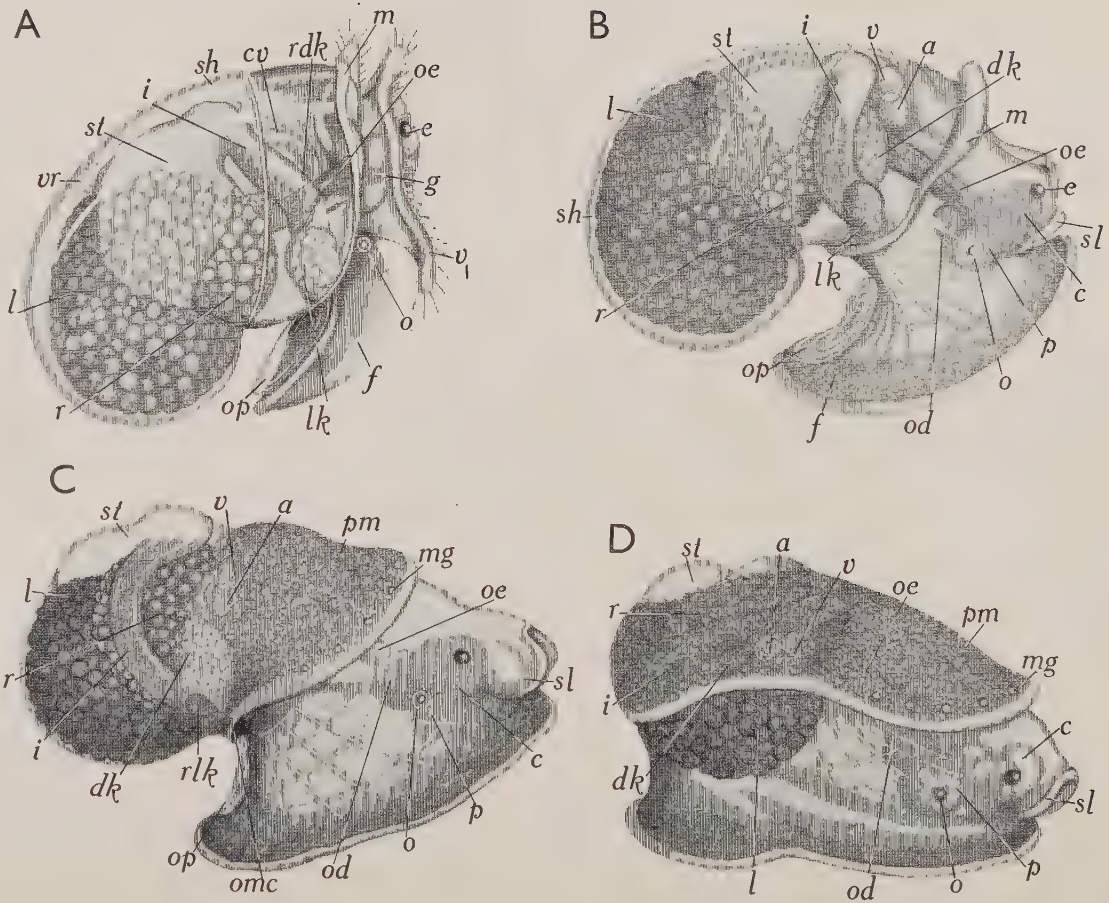
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Fig. 5.

A. Veliger from the right side: × 170.

B. Early post-veliger: × 150.

C. Embryo at mid-detorsion: × 120.

D. Embryo near completion of detorsion: × 110.

a, auricle; *c*, cerebral ganglion; *cv*, cardiac vesicle; *dk*, definitive kidney; *e*, eye; *f*, foot; *g*, ganglia of nerve ring; *i*, intestine; *l*, left lobe of digestive gland; *lk*, larval kidney or secondary kidney; *m*, mantle; *mg*, marginal gland; *o*, otocyst; *od*, odontophore; *oe*, oesophagus; *omc*, opening of mantle cavity; *op*, operculum; *p*, pedal ganglion; *pm*, pigment of mantle; *r*, right lobe of digestive gland; *rdk*, rudiments of definitive kidney; *rlk*, remains of larval kidney; *sh*, shell; *sl*, sensory lobe; *st*, stomach; *v*, ventricle; v_1, velum; *vr*, velar retractor muscle.

the adult, and runs straight back to the stomach (*st*), into which it opens ventrally on the right side. The stomach and digestive gland comprise the visceral mass, the latter not yet constricted from the stomach. The gland is divided into two unequal lobes—a larger left one (Figs. 5A and 6A, *l*) which occupies the whole left wall and spreads posteriorly and ventrally around the gastric sac, and a smaller right lobe (*r*) which is restricted to an anterior strip on the right wall. The intestine (Fig. 5A, *i*) leaves the stomach dorsally, on the right side, above the point of entry of the oesophagus, and passes below the dorsal integument to the anus. The heart is dorsal and towards the right side: in the early veliger it is indicated only by a vesicle (Figs. 5A and 6A, *cv*) which contracts at rather irregular intervals; but later the auricle and ventricle can be distinguished, the latter lying to the left and slightly posterior to the auricle. The velar retractor muscle (Fig. 5A, *vr*) is situated on the left side of the larva and is inserted anteriorly around the foregut and the posterior part of the velum. It passes back on the left side of the stomach to its posterior attachment near the median part of the shell. The velum is withdrawn into the shell when the muscle contracts—a movement which will also help the circulation of the blood.

The organization of the veliger is typically prosobranch, as is indicated by the following points: the mantle cavity lies on the right side, the rudiments of the definitive kidney are anterior to the rectum, and it is the hepatic area on the left which is enlarged.

The Post-veliger

During early post-veliger life (Fig. 5B) the foot (*f*), now covered by ciliated epithelium except in the region of the operculum, develops a large, oval, creeping sole which enables the embryo to cling to and creep around the capsule wall, the path being lubricated by the pedal mucous gland. The otocysts (*o*) persist and Joyeux-Laffuie (1882) has traced them into the adult; in the later stages of development, at the time when the operculum is lost, they are far less obvious. The velum is not cast off as a whole, its ciliated covering is gradually lost and the two halves appear to be transformed into the sensory lobes which border the mouth (Figs. 5 B, C, D, *sl*) and have been described in the adult. The tentacles are still represented by the broad bosses with an eye situated in the middle of each. Later the shell (Fig. 5B, *sh*) is cast off and the velar retractor muscle lost.

When detorsion takes place it is effected slowly, and, as may be seen from Figs. 5 B, C and D, it is accompanied by an increase in the diameter of the neck region connecting the head and foot to the visceral hump, mainly in an antero-posterior direction, and also by the gradual recession of the visceral hump as the viscera come to project into this neck. While the mantle cavity migrates along the right side to a more posterior position its opening to the exterior is reduced in size; the blind end, which receives at its extremity the opening of the rectum, and is at first directed posteriorly, becomes ciliated. Black pigment is developed in the subepithelial tissues of the mantle (Fig. 5 C, D, *pm*), appearing at first around the anterior border and immediately behind the rudimentary marginal glands, which are just visible, and then extending laterally so as to reach the posterior end, leaving the visceral hump light in colour because here the mantle is thin and there is little or no subepithelial tissue. As the visceral mass is reduced in size so the mantle which covers it thickens and then becomes pigmented.

During detorsion the stomach and digestive gland rotate through approximately 90°, so that the right side becomes dorsal and the dorsal surface lies towards the left. It must be emphasized that this movement is not one of detorsion, but is forced upon the alimentary canal so that it can accommodate itself to changes in the disposition of viscera elsewhere, due to movements of detorsion. The left lobe of the digestive gland (Fig. 5 C, D, *l*) becomes ventral and the smaller right lobe dorsal (*r*). They have both been gradually constricted from the stomach and are now completely separated from it at the posterior end, where it forms a closed sac lined by columnar epithelium. The original left lobe opens into the stomach by an aperture occupying the greater part of the ventral wall (Fig. 6B, *vo*), and the original right one has a dorsal and less extensive opening into the anterior end and towards the right side (*do*). Each extends forwards for a short distance in front of the stomach, and the oesophagus is now directed dorsally over the broad anterior diverticulum from the ventral lobe, and opens into the stomach between this and the small anterior diverticulum from the dorsal lobe. The intestine, lined by ciliated epithelium, still arises dorsally, but on the left side (*oi*), a short distance behind the oesophageal opening. It passes over the oesophagus, as in the adult, to describe a slight anterior loop, and then leads back on the right side, behind the definitive kidney, to the anus (Fig. 5 C, D). During its whole course it lies dorsal to the digestive gland and at times the beating of the cilia of the epithelium and the sudden contraction of muscle cells, which are developing around the intestinal wall, can be seen through the mantle.

In the early stages of detorsion there are only two openings of the digestive gland into the stomach, one for each lobe. Later a third appears at the posterior end of the stomach. It is ventral (Fig. 6C, *po*) and communicates with the posterior portion of the original left liver lobe, which has become divided from the anterior part and forms the posterior liver lobe of the adult. The writer cannot agree with Joyeux-Laffuie's (1882) statement that the posterior lobe of the adult is derived from the original right: here, as in *Aeolis* (Fischer, 1892), it is the larger left lobe which divides to give the left and posterior lobes of the adult.

By this time the cilia around the mouth have been lost and the radula is developed. As soon as the odontophore muscles are functional the tongue is, at intervals, rhythmically protruded through the mouth; at first the action is only feeble, but later the radula draws in some of the surrounding albumen which is used as food. The salivary glands are distinguishable in the late veliger, when they appear as clumps of mucous cells pressed against the oesophageal tube and behind the nerve ring, one clump lying against the ventral wall of the oesophagus and the other against the dorsal. With detorsion the oesophagus rotates through 90° so that the ventral clump of mucous cells becomes the left salivary gland of the adult, and the dorsal group the right salivary gland.

The secondary kidney (Fig. 5C, *rlk*) degenerates as the definitive kidney (*dk*) is differentiated. The latter forms a rather elongated mass of cells, one end of which lies on the median side of the larval kidney and abuts on to the mantle cavity, whilst the other end is directed dorsally. The kidney is in communication with the pericardium by the renopericardial duct, which at this stage is relatively much wider and shorter than in the adult and is not ciliated. It is given off from the auricular end of the pericardium and runs towards the mantle cavity. At the point where it reaches the ventral end of the definitive kidney it opens into the primary ureter which discharges into the mantle cavity in front of and dorsal to the anus. It is thus seen that whereas in the typical mollusc the excretory tissue is developed on the walls of the original coelomoduct, connecting the pericardium and mantle cavity, in *Onchidella* these remain always relatively undifferentiated, and the definitive excretory cells are formed from the block of tissue lying alongside the duct. At a later stage this block of tissue cavitates and the lumen is placed in connexion with the pericardium by way of the renopericardial canal, and with the mantle cavity by means of the primary ureter. The heart is at first transverse and the auricle lies to the right of the ventricle and in front of the anterior loop of the intestine. During detorsion it moves back along the right side and the auricle comes to lie behind the ventricle (Fig. 5B, D, *a*, *v*).

The Embryo at the time of Hatching

Some days before the embryo frees itself from the capsule it has attained the superficial appearance of the adult: it is oval in outline, a flat creeping foot covers the ventral surface and the pigmented mantle covers the dorsal. All traces of the projecting visceral hump have disappeared. The tentacles are still broad lobes bearing the eyes and they are invaginable. The head can be withdrawn into the shelter of the mantle which has grown down over it, and also overhangs the foot, so that the side walls of the body (Hoffmann, 1929) are hidden. As the mantle edge grows downwards these side walls rotate from their original vertical position to one where they slope upwards and outwards at an angle of 45°. The edge of the mantle turns under and inwards to join them and the two surfaces lie in the same plane. The marginal glands (Fig. 5D, *mg*), developed in the tissues of the thickened edge of the mantle, open on to the dorsal surface; as yet no papillae are developed. The opening of the mantle cavity (Fig. 6C, *omc*) has the appearance of a small

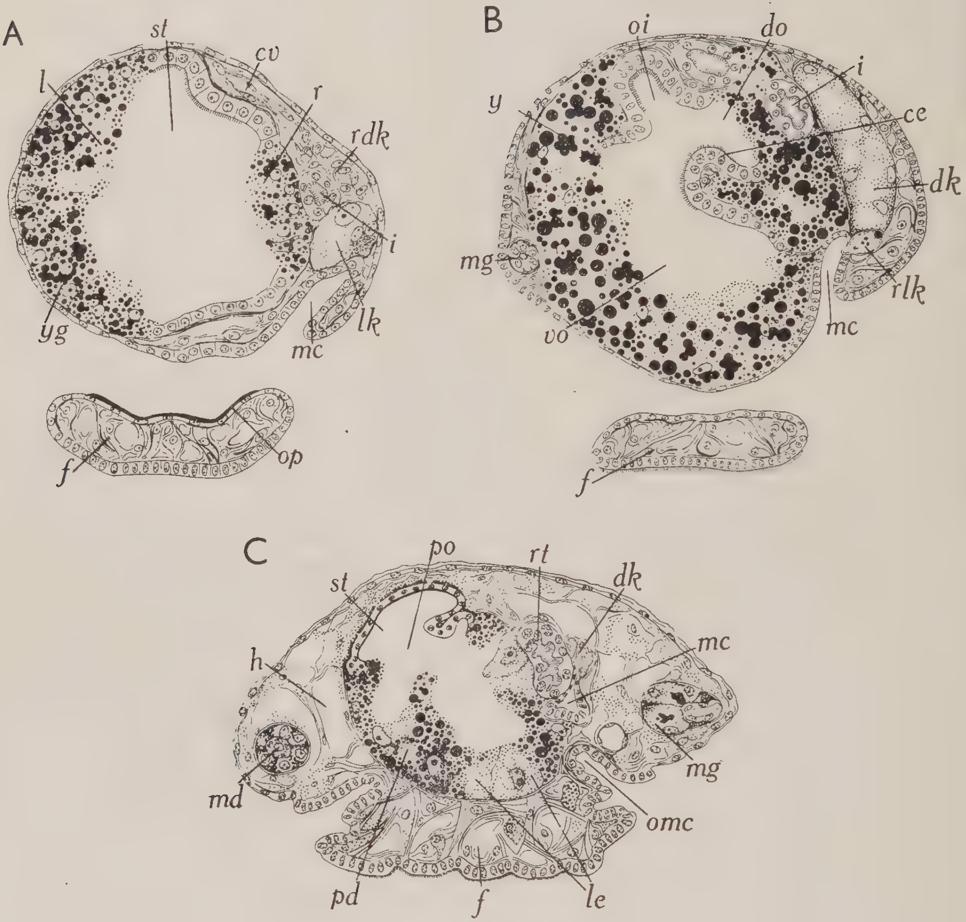
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Fig. 6. A. Veliger. Transverse section through stomach and larval kidney: ×240. B. Embryo at mid-detorsion. Transverse section through stomach and kidney: ×230. C. Embryo at time of hatching. Transverse section to show kidney opening into tubular mantle cavity and opening of posterior lobe of digestive gland: ×207. *ce*, ciliated epithelium of stomach; *do*, opening of right lobe of digestive gland; *h*, haemocoelic space; *le*, lime and excretory cells forming; *mc*, mantle cavity; *md*, duct of marginal gland; *oi*, origin of intestine; *pd*, posterior lobe of digestive gland; *po*, opening of posterior lobe of digestive gland; *rt*, rectum; *vo*, opening of left lobe of digestive gland; *yg*, yolk granules. Other letters as in Fig. 5.

round hole at the posterior end of the body, between the foot and the mantle, and to the right of the middle line. The heart is in the adult position, on the right side and posterior, the auricle lying behind the ventricle, and the pulsation of the two chambers can be seen through the mantle.

The capsule wall is gradually weakened by the action of the radula, perhaps assisted by an enzyme, and as soon as it is punctured the embryo emerges. It is difficult to find these young animals in the sandy rock crevices. Only three have been seen in their natural habitat and these were creeping over the egg masses from which they had just emerged. They do not appear to cluster with the rest of the community in the crevice, for the smallest individuals found in any community were 2·6 mm. in length and showed the characteristics of the adult, whereas the newly hatched forms are 1·2 mm. in length, and in them neither lung nor reproductive organs are formed and the kidney is a simple, unbranched sac lying on the right side posteriorly.

The alimentary canal shows all the features of the adult. A jaw is developed in the roof of the buccal cavity and behind this the dorsal ciliated channel runs back to the oesophagus; the rest of the buccal cavity is lined by a cuticle. Before passing through the nerve ring the oesophagus receives the openings of the salivary glands which have now migrated through the nerve ring; as yet no salivary duct is developed. The epithelium of the crop is covered by a cuticle, and the crop is closed from the stomach by a sphincter. Because of differential growth of the stomach wall the anterior liver openings, which occupied a dorsal and ventral position during detorsion, are now gradually regaining their original orientation of right and left, and are opening into the initial chamber. At this stage they are wide, with no duct. Behind them the triturating stomach is differentiated, the epithelium being covered by a thickening cuticle beneath which the dorsal and ventral plates are formed. The posterior liver opening is still ventral (Fig. 6C, *po*). The lobes of the digestive gland are branching to form the initial tubules, and in the crypts of these lime cells and excretory cells are forming (*le*). The body cavity is approximately circular in transverse section and bounded on each side by a muscular diaphragm; it is filled for the most part by the digestive system. The greater part of the digestive gland lies ventral to the gut and has not as yet spread over the intestine. This has lengthened and assumes the coilings of the adult, though the ventral caecum of the anterior intestine and the typhlosole cannot be distinguished. At the posterior end of the gizzard, and on the right side, the rectum (*rt*) passes ventrally to open into the mantle cavity (*mc*). This is a short tube which runs dorsally to the anus, and is ciliated except around its opening to the exterior (*omc*), where it is surrounded by a sphincter. To the right of the anus it sends a small diverticulum to the opening of the definitive kidney (*dk*), and from this is derived the secondary ureter. In the adult this cavity has previously been called the rectum and its opening to the exterior the anus, but from its development it is clear that it is a reduced mantle cavity, and this explains the fact that both the alimentary canal and the kidney open into it (Fig. 1B, *mc*), and that its opening is connected to the vaginal opening by a groove, the two being surrounded by common lips. The kidney is still a compact, pear-shaped mass, with a small central cavity, lying on the right of the rectum just behind the heart; the broader end is dorsal and the ventral end opens into the incipient secondary ureter. The heart and kidney are separated from the general body cavity by the right diaphragm. It is not until later in development that the kidney branches and spreads around the posterior wall of the rectum to the left side of the body, where it penetrates the haemocoelic spaces (Fig. 6C, *h*) between the left diaphragm and the body wall. Carried round by this growth the secondary ureter comes to open into the posterior wall of the mantle cavity (Fig. 1B, *su*) instead of its right side, and into it the primary ureter projects as a long papilla (*u*) which is slung from the walls by two lateral sheets of tissue. A small mass of undifferentiated cells which will give rise to the reproductive organs lies posterior to the stomach. No lung has yet been formed, and it would seem that as it opens separately from the mantle cavity it must arise as a separate and independent invagination of the mantle epithelium which grows inwards and forwards to come into intimate contact with the haemocoelic blood spaces around the kidney.

The smallest individual which has been seen in any adult community measured, as previously stated, 2·6 mm. in length. It was found during mid-August and was presumably hatched from eggs laid in the previous season. The mantle cavity was relatively wide and still opened on the right side of the body, receiving just within the mouth of the opening, and anterior to the anus, the female aperture, and posteriorly the opening of the ureter. From the kidney a small diverticulum had spread around the posterior end of the body and penetrated the haemocoelic spaces on the left side. The pneumostome was posterior and to the right of the opening of the mantle cavity, and led through a short duct to a small and comparatively unbranched pulmonary sac. This was in contact with the kidney on the right side, and a tendency to spread around the posterior diverticulum was indicated.

Discussion

The mantle cavity of the typical mollusc contains a definite series of organs and into it opens a definite number of apertures. The structure of the veliger of *Onchidella celtica* is essentially comparable with that of a monotocardian prosobranch, except that no ctenidium and hence no osphradium are present, so that if it possesses a cavity homologous with the molluscan mantle cavity we should expect to find opening into it a single kidney, the anus and the genital aperture. The cavity which has already been described on the right side of the veliger agrees with this, except that no genital aperture has yet appeared. The cavity can therefore be taken as a true mantle cavity. During the metamorphosis of the larva into the adult the rotation due to detorsion rotates it from its original position on the right side of the body to a median position posteriorly, carrying the anus and kidney opening with it, and meanwhile it becomes converted into a narrow tubular space with its opening contracted into a small pore. The kidney spreads posteriorly around the mantle cavity and on to the left side of the body, bringing about the consequent migration of its opening into the mantle cavity which has already been described. The net result of all these changes is that at the end of metamorphosis the animal has a secondary bilateral symmetry, a type of reorganization which is of frequent occurrence in detorted gastropods accompanying the loss of shell and reduction of the visceral hump. The median opening at the posterior end of the body of *Onchidella*, which has previously been called the anus, is thus seen to be nothing more nor less than the opening of the mantle cavity in its new position, and what has been called the secondary opening of the excretory system into the rectum is nothing but a retention of the original opening of the kidney into the mantle cavity, as in all molluscs. The former interpretation of the relationship of these parts was obviously possible only so long as the real nature of the cavity was unknown. In the adult animal the female aperture also opens into the mouth of the mantle cavity and on its right side, providing a further clue as to the identity of this space. As the penis lies on the side of the animal's head anterior to the region affected by detorsion, it retains this position in the adult.

Since it is clear that the space into which the digestive, excretory and genital systems open is the homologue of the mantle cavity of other molluscs, then the cavity called the lung—the possession of which is one of the main reasons for regarding the Onchidiidae as pulmonate gastropods—must be a new structure, independently evolved and not homologous with the lung of the pulmonates. This being so the question now arises as to whether the Onchidiidae should not be regarded as opisthobranchs which have acquired a pulmonary cavity. Both Plate (1893) and Hoffmann (1929) would agree that whatever the anatomical specialization of the genus *Onchidella* may be it is at least typical of the Onchidiidae as regards its habitat; were the Onchidiidae, as Bergh (1885) suggests, true pulmonates they must be regarded as attempting to invade the sea from the land: on the former view the direction of migration would be reversed.

The opisthobranch affinities of the group have previously been recognized by Plate (1893) and Labbé (1934). The former assumes a tectibranch origin for the pulmonates and proposes to derive the Onchidiidae from the base of the pulmonate stem; the latter actually regards them as a group of opisthobranchs. The most important points in which these molluscs agree with the opisthobranchs are: (1) the reduction in size of the visceral hump; (2) the loss of the shell; (3) the posterior position of the mantle cavity; (4) the position of the auricle behind the ventricle; (5) the reduction of ganglia in the visceral loop to three; (6) the possession of three liver lobes, two anterior and one posterior; (7) the general disposition of the reproductive organs; (8) the veliger larva with its primary and secondary kidneys. On the other hand, it may be said with Bergh (1885) that the Onchidiidae possess a number of characters in common with the pulmonates, the most important of which are: (1) the possession of a lung; (2) the shortening of the visceral loop; (3) the retractile tentacles bearing eyes at their summits; (4) the loss of a seminal groove; (5) the development of thick chitinous plates in the stomach; (6) the number of lateral teeth on the radula. The occurrence of these features may, however, be explained in other ways: the lung in the two groups is not homologous; the shortening of the visceral loop is not confined to pulmonates, but is found in many nudibranchs; the development of eyes on the tentacles is indicated even in the prosobranch molluscs such as *Buccinum* and *Nucella*, though here the tentacles are not invaginable; in dorids the seminal groove is lost and these nudibranchs have also numerous lateral teeth on the radula. The stomach of the Onchidiidae is built on the typical prosobranch plan in that the crushing plates are developed in the stomach itself, and differs from that of the tectibranch where the plates are oesophageal and the stomach is reduced (Fretter, 1939). Its distinguishing features can be directly attributed to the quantities of sand which are ingested with the food (Heidermanns, 1924).

It is generally recognized that the opisthobranchs are derived from monotocardian prosobranchs. The pulmonates and the opisthobranchs have retained only one set of the organs associated with the mantle cavity and so may have come either from closely related prosobranch groups or the pulmonates may have originated from an early group of the opisthobranchs themselves. If the latter be the case, then it may be suggested that the Onchidiidae separated from the opisthobranch stem at about the same time. From this origin, however, the Onchidiidae have evolved along different lines from the pulmonates, retaining more opisthobranch characters. Owing to adaptation to similar habitats they have acquired a superficial resemblance to the nudibranchs, and the acquisition of a lung, a structure independent of the true mantle cavity, has enabled them to become most active during intertidal periods, and so to exploit a habitat at a time when it is closed to other littoral gastropods. Even with this organ of terrestrial respiration their range is still restricted by their dependence on a relatively high humidity, although it is doubtful how serious this factor would be in limiting activity on the seashore. Many of the features which the Onchidiidae share with the pulmonates may be attributed to the close origin of the two groups, the similarity of their diet and their air-breathing habit. The pulmonary respiration of *Onchidella celtica* is by itself insufficient for the respiratory needs of the animal, but is a necessary supplement to pallial respiration when the animal is active.

The distribution of the Onchidiidae, the northern limit of which is the extreme south-western coasts of England, and likewise their inactivity during the winter months, suggest that they are unable to endure a low temperature. The tendency towards the adoption of a terrestrial mode of life has been intensified in other members of the group: some live above the intertidal zone and *Onchis montana* is definitely terrestrial.

On the whole it would seem that the most suitable place to assign the group is with the opisthobranchs, regarding it as a small twig from the base of the main stem. This is in agreement with Labbé (1934), though he prefers the name Silicodermatae to Onchidacea. He states that in *O. celtica* spicules of silica are found in the connective tissue under the epidermis, the largest measuring $25\,\mu$ and occupying a large part of the dermal tissue. In other species he describes the spicules as being much larger. No suggestion of such spicules has been found in *O. celtica*, nor could they be identified in the examination, which was kindly made by Mr A. G. Lowndes, of the nota of forty newly collected specimens. Unless the spicules are much more conspicuous in the other genera, the name Silicodermatae would appear to be a misnomer.

Mr Lowndes has given me the following note:

> The material given to me was weighed, 3·397 g. It was placed in a Kjeldahl flask together with about 100 ml. of concentrated sulphuric acid and a sufficient quantity of potassium sulphate. After boiling up the mixture for an hour a drop of mercury was added and the whole again boiled till the liquid in the flask became quite clear and colourless. When the contents of the flask were cool they were washed out into a 1000 ml. beaker and allowed to remain overnight. A small amount of sediment was to be seen on the bottom of the beaker. Nearly all the liquid was poured off and about 50 ml. of concentrated nitric acid added. The beaker was then filled with distilled water and allowed to stand for 4 hr. There was a small amount of sediment and it was examined in water both in a watch-glass and on a slide under a Zeiss D objective. The sediment was seen to consist of a few sand grains, a few identifiable sponge spicules and a very large number of diatom frustules. The length of the diatoms was found to be $8\,\mu$ in most cases. There was also a small amount of matrix consisting of amorphous silica but no recognizable spicules other than those mentioned were found. [*A. G. Lowndes.*]

SUMMARY

Onchidella celtica lives in communities in intertidal rock crevices, emerging only when uncovered by the tide during the milder months (March–November). It feeds on young algae and diatoms, rasping them from the rock with the radula which is lubricated by a mucous saliva. The distensible crop and stomach afford ample storage space for the food. The stomach consists of three chambers (Fig. 2), and the dorsal (*d*) and ventral (*v*) chitinous plates of the posterior one, the gizzard, crush the contents and help to direct the soluble portion towards the ducts of the digestive gland. There are three of these—the two anterior (*ld*, *rd*) are large and lead from the initial chamber of the stomach (*ic*), the third (*pd*) is small and joins the posterior wall of the gizzard (*pw*). The digestive gland secretes into the stomach; no cellulase occurs. Feeding experiments suggest that the digestive cells of the gland absorb only fluid. Lime and excretory cells occur in the crypts of the digestive epithelium, the latter concerned with taking up excretory matter from the blood and elaborating it into spherical masses for evacuation with the faeces. From the anterior ducts this waste matter, and that from the

digestive cells, is directed to a small caecum (*c*), and so prevented from mixing with food in the stomach, and there is consolidated and cemented to the faecal rod which fills the intestine a few hours after a meal. The large quantity of indigestible and undigested food in the stomach is directed into the intestine by the mechanical action of the gizzard.

The mantle is solely responsible for respiration when the mollusc is covered by water or is inactive at low tide. At other times pulmonary supplements pallial respiration—the pneumostome (Fig. 1 A, *p*) is widely open during periods of activity and the cavity of the lung distended. The lung follows the course of the kidney; its walls are produced into fine diverticula which penetrate the nearby haemocoelic spaces (Fig. 3 A), and the respiratory surface consists of parallel blood channels lined by squamous epithelium (*os*, *is*, *sc*). *O. celtica* will survive in an atmosphere of 90% R.H. for three days without apparent effect.

The period of sexual activity extends over the summer months; egg capsules are laid during July, August and the beginning of September. The upper part of the hermaphrodite duct acts as a vesicula seminalis and here there is evidence of sperm absorption. Distally the duct divides into two channels, the oviduct (Fig. 4, *ov*) and vas deferens (*vd*), which are glandular and share a common dorsal wall. Both are ciliated and the gland cells are subepithelial. At the beginning of the vas deferens a large diverticulum forms the prostate (*pr*). The upper part of the oviduct bears six diverticula (I–VI) along which the eggs must travel. The first of these, the smallest, overhangs the fertilization chamber (*fc*) and acts as a receptaculum seminis (*rs*); the other five are of increasing size, and the sixth is wound into a spiral. The fertilization chamber receives the ducts of the albumen gland (*dag*). The two channels of the genital duct become entirely separated: the vas deferens forms a narrow non-glandular tube (vd_1) and follows the external ciliated groove (Fig. 1 A, *cg*) on its way to the penis; the oviduct becomes less glandular and at the beginning of the muscular vagina (Fig. 4, *v*) it receives the duct from the bursa copulatrix (*dbc*) and, approximately opposite this, the duct of a glandular flagellum (*fl*).

The egg capsules are cemented to the walls of the rock crevices. Each is an elongated tube distended at regular intervals by an egg and coiled in a mucoid secretion. Each egg is surrounded by albumen, and by a capsule wall made of three distinct layers; the consistency and staining properties of these suggest that they are secreted by the diverticula of the oviduct (I–VI). The embedding mucoid secretion is probably produced by the pedal mucous gland (Fig. 1 A, *dc*, *vc*). Whilst in the egg capsule the egg develops into a typical prosobranch monotocardian veliger (Fig. 5 A) which undergoes detorsion and assumes the external appearance of the adult (Fig. 5 B, C, D). At this stage it hatches. There is no lung and the kidney is a simple sac behind the heart. The lung appears to develop not from the mantle cavity, but as a separate invagination of the mantle epithelium behind this cavity.

The embryological study shows that the tube which has previously been called the rectum is a reduced mantle cavity receiving the openings of the anus, the ureter and, at its mouth, the vagina. The new interpretation of the homologies of these parts calls for a revision of the systematic position of the Onchidiidae; its affinities with the prosobranchs are discussed and it is concluded that the family should be regarded as an early offshoot from the main stem of the opisthobranchs.

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THE LARVAE OF THE GENUS *PORCELLANA* (CRUSTACEA DECAPODA) AND RELATED FORMS

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(Text-figs. 1–12)

Although the larva of *Porcellana* is one of the earliest known decapod larvae and at a later date the different stages of *P. longicornis* were well described by Sars (1889), there is yet much that is unknown. Few larvae have been actually hatched from the egg and in no case has the number of larval stages been definitely ascertained. In order to fill in some of the gaps in our knowledge a detailed study of the two common Plymouth species was begun (*P. longicornis* and *P. platycheles*). Unfortunately, war conditions made it difficult to obtain material and many specimens were lost as the result of air-raids; it was therefore not until the summer of 1942 that the work could be finished.

It is now possible to distinguish between the two species in all stages. Both have been hatched from the egg, larvae from the plankton have moulted and the post-larvae have been obtained from the last larvae. A comparison with Gurney's (1938*a*) notes on the species from the Red Sea and his general comments on the larvae of the genus and its relatives (1942) is of much use. These, with the first larvae of *Petrolisthes armatus* hatched by myself at Bermuda and certain larvae described by other workers, have made it possible to make some progress in the elucidation of the relationships of the larvae.

Porcellana longicornis and *P. platycheles* are both common at Plymouth; but whereas the latter is confined to coastal waters and lives between tidemarks and down to about 3 fathoms, the former has a much wider range and although occurring with *P. platycheles* between tidemarks extends to well beyond the Eddystone grounds. It was thought that a third species was present in the outside waters (see Gurney, 1942), but this larva has now been proved to belong to *P. platycheles*.

Large numbers of *Porcellana* larvae are caught in the tow-nets from between the Breakwater and the Rame-Eddystone grounds. It has always been assumed that these represented the two species. All the late larvae, with the exception of a very few specimens, rarely obtained, possessed three pairs of pleopods and this fact gave rise to the assumption that both species had only three pairs. The few specimens with four pairs of pleopods were regarded as a new species. It is now certain that all those with three pairs of pleopods belong to *P. longicornis* and the few rare specimens with four pairs belong to *P. platycheles*. The fact that under present conditions tow-nettings were unobtainable beyond the Breakwater led to the detailed study of the plankton of the inshore waters—hitherto rather neglected for the richer outside hauls. Larvae of *P. platycheles* (recognized from comparison with those hatched from the parent) were collected in these hauls and moults from the first to the second stages obtained, proving without a doubt that *P. platycheles* has four pairs of pleopods and is identical with the form rarely present in the outside hauls. The larvae of *P. platycheles*, however, are never plentiful even in the inshore samples, probably because of their large size and because only small tow-nets can at present be used. Larvae of *P. longicornis* are more abundant, but they are not caught in large numbers. It is samples taken with the 2 m. ring trawl at Station L_4 and beyond, which at times are so full of the larvae of *P. longicornis*.

P. longicornis breeds from January to November, the larvae being most abundant in spring and summer. *P. platycheles* has a shorter breeding season beginning in April or May (very rarely March) and finishing by September, June to the end of July being its usual maximum breeding period.

Sars (1889) has described the larvae of *P. longicornis* in detail; he found two distinct stages in the plankton, but suggested that the earliest was not the first—a suggestion which is supported by hatching the larvae at Plymouth. A later stage than his last also occurs at Plymouth although the post-larvae obtained all moulted from the less developed form similar to his last. His description of the two stages of *P. longicornis* may be taken as typical of the two main stages of a Porcellanid larva. As is shown below, however, there are three or four stages (possibly five) in *P. longicornis* and two (possibly three) in *P. platycheles*.

In all Porcellanid larvae known there are two main stages (I and II). Stage I is exemplified by Sars's earliest stage, in which the exopods of the first and second maxillipedes bear 4 setae and the telson 7+7 setae, the third maxillipede and legs being rudimentary and very small. Stage II is exemplified by Sars's last stage, in which the exopods of the first and second maxillipedes bear 10–12 setae, that of the third either having setae (usually 4) or not, the endopod and also the legs being non-functional, but more developed. The telson either has 8+8 setae (*Porcellana*) or 7+7 with a central tooth (*Petrolisthes*). The first of the main stages may include two, called here I*a* and I*b*, and the second may include two or three, called here II*a*, II*b* and II*c*. Gurney (1938*a*) found only two stages in *Porcellana inaequalis* and *Petrolisthes*? sp. from the Red Sea. Menon (1937) concluded that there were probably three in his Indian Porcellanids.

The young is hatched as a pre-zoea (Fig. 1*a*) which is much the same in all the species in which it is known. It lives for some hours, coming up to the light and to the surface and swimming vigorously. The antennule has two large feathered apical spines with aesthetes showing at the base, and may or may not have a lateral spine (Fig. 1*b*). The antenna (Fig. 1*c*) has 5 large spines covering the zoeal exopod and a single spine over the endopod (*Porcellana longicornis, P. platycheles* and *P. inaequalis*) or 6 over the exopod in a species from Samoa described by Gurney (1926, 1942) in which there is also a lateral spine on the endopod. The telson has 6+6 spines, the first small and smooth, the others long and feathered. There is no trace of the second zoeal spine until just before the pre-zoeal cuticle is shed, when it may be present as a minute rudiment. The small posterior spine of the zoea internal to the seventh spine is included in the sixth pre-zoeal spine (Fig. 1*d*;

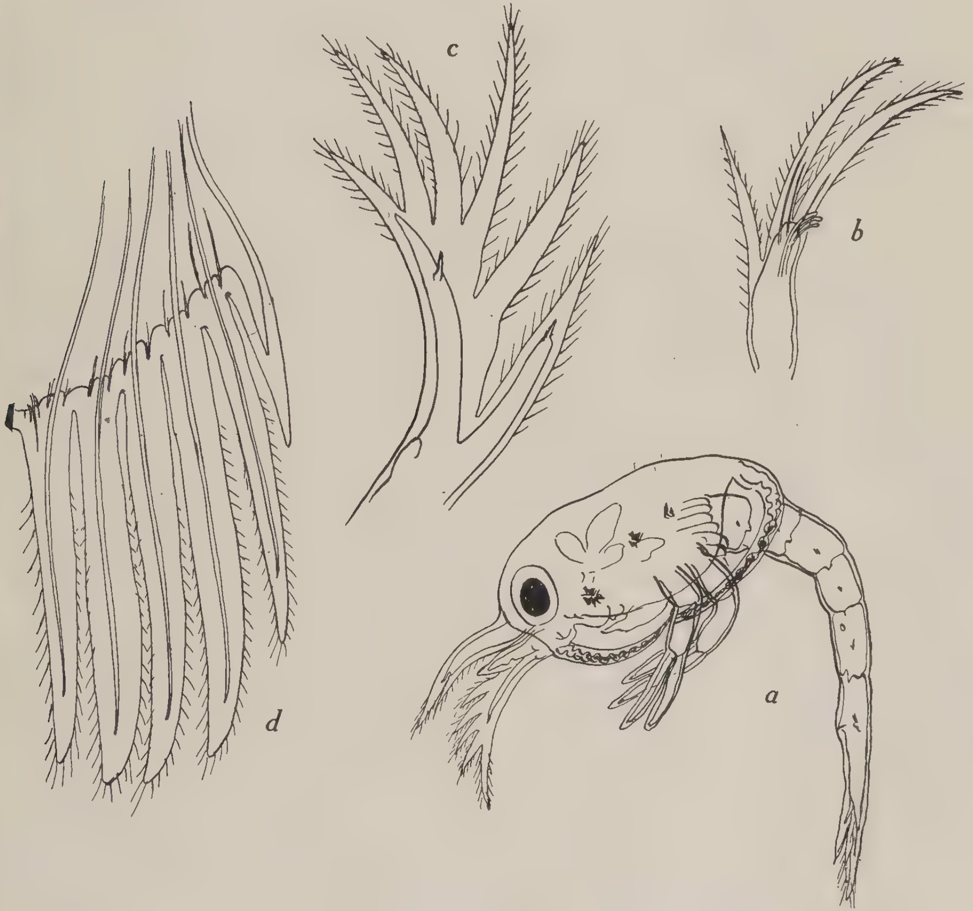

Fig. 1. Pre-zoea of *Porcellana platycheles*: *a*, side view, 2·4 mm. long without long spines; *b*, antennule; *c*, antenna; *d*, telson.

see also Gurney, 1942, p. 53, fig. 14*a*). The rostral and posterior carapace spines of stage I are crumpled up under the pre-zoeal cuticle.

The pre-zoea moults into stage I, either *a* or *b* (*a* in *P. longicornis*, *b* in *P. platycheles*). The zoea in all stages has a very long rostral spine and the posterior angles of the carapace are drawn out into long spines, the posterior carapace spines. The telson is triangular and bluntly pointed posteriorly. The second spine is hair-like. There is a small spine on each side posteriorly internal to the fifth seta. An anal spine is present. Uropods are absent and there are only 5 abdominal somites which may or may not have lateral spines. [A curious larva described by Gurney (1924*c*, 1942) from the *Terra Nova* Expedition and possibly not a true Porcellanid, is exceptional in possessing uropods in stage II, the telson deeply forked and an antennal scale.] The antennule is a simple rod armed with aesthetes and setae at the tips. In stage II segments may show through the cuticle and there may be groups of hairs to mark the segments. The antenna has a simple rod-like exopod and endopod without setae, but usually there are a few minute spines near the tip of the endopod and sometimes a hair-like spine on the exopod. In the latest stages the segments of the post-larval flagellum show through the cuticle. The mandible is without a palp (except in the form identified by Faxon (1879) as *Polyonyx*, which has a rudimentary one). The maxillule has an unjointed endopod with 3 setae at the tip and well-defined setose inner lobes. The outer margin bulges slightly in the region where the exopod should be. The maxilla has an unjointed endopod with 3 terminal and, usually, 2 pairs of inner setae and 4 well-defined inner setose lobes; the exopod has a long posterior lobe bearing one large seta posteriorly and 5 or 6 setae round the anterior and outer margin in stage I, more in stage II. The first and second maxillipedes are functional with 4 setae on the exopod in stage I, and 10–12 setae in stage II, and an endopod of 4 segments with no external setae on segments 1–3 in stage I, these being present in stage II. There are very small rudiments of the third maxillipede and legs in stage I, but the former is bilobed. In stage II the endopod is large, but not functional, the exopod either being unarmed (*Porcellana inaequalis*, *Petrolisthes* sp. (Gurney, 1939*a*)) or bearing setae (4 in *Porcellana longicornis* and *P. platycheles*, 3, 4 or 6 in Porcellanids described by Menon (1937)). The legs are non-functional, the fifth being curved forward under the others and chelate. There are no pleopods in stage I, but there may be 3 or 4 pairs in stage II. These are never setose, but are long in the stage (II*b* or II*c*) which changes to the post-larva. The post-larva differs markedly from the adult and certain characters have not yet appeared. There are now six abdominal somites and setose uropods; 3 or 4 pairs of setose pleopods are present. The telson is a plain plate. The legs are functional and the antennal flagellum long.

The number of larval stages varies in different species and even in the same species, but stages I and II are always present. Stage I may consist of two stages (I*a*, I*b*) or of one stage only (I*b*), stage II may consist of three stages (II*a*, II*b*, II*c*) or two (II*b*, II*c*) or of one only (II*b*). It is impossible to be certain that there is a moult between I*a* and I*b* or between II*b* and II*c* and a very wide range of individual variation is indicated. It is probable that the normal life-history of the genus is I*b*–II*b*–post-larva, as in *P. platycheles* (II*c* also being present as an alternative to II*b*) and possibly others, and these are more or less stable, but in *P. longicornis* (I*a* or *b*–II*a*–II*b* or *c*–post-larva) there exists a state of great variability not as yet settled down to normal.

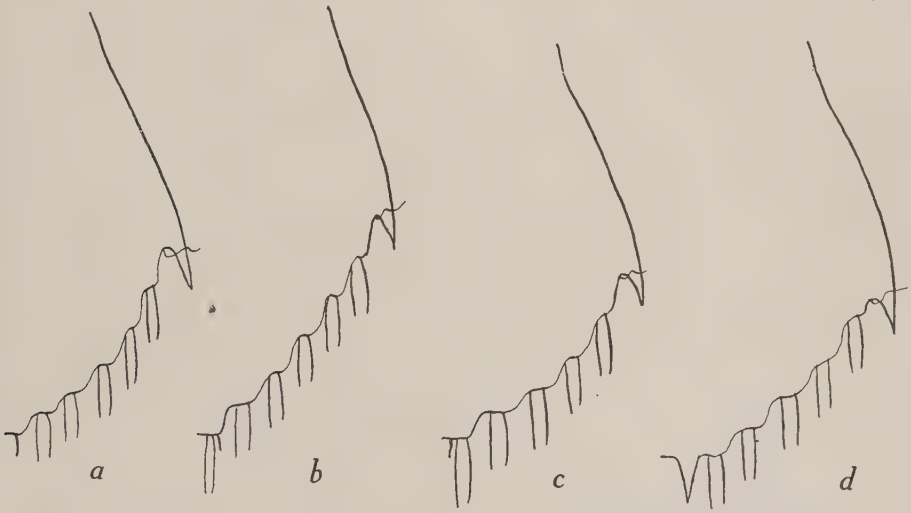

Fig. 2. Diagram of telson of *Porcellana* and *Petrolisthes*: *a*, *Porcellana longicornis*, stage I; *b*, *P. longicornis*, stage II; *c*, *Petrolisthes armatus*, stage I; *d*, *Petrolisthes* sp. (after Gurney), stage II.

Gurney (1938*a*) has shown that there are 4 pairs of pleopods in the larvae of *Porcellana inaequalis* and in *Petrolisthes* sp., and it is now found that there are also 4 pairs in *Porcellana platycheles*; therefore, as he truly remarks, this is not a generic character as there are 3 pairs in *P. longicornis*. He has also shown that *Porcellana* and *Petrolisthes* have a different type of telson respectively. *Porcellana* in stage I has the fifth long seta situated outside the central prominence, *Petrolisthes* has it on the central prominence (Fig. 2). In stage II *Porcellana* has a sixth long seta on the central prominence, whereas in *Petrolisthes* there is no sixth seta but a central tooth. As *Petrolisthes armatus* from Bermuda, moulted from the pre-zoea hatched from the egg, has a similar type of telson to Gurney's *Petrolisthes* it may safely be assumed that he is right in attributing his form to that genus. The type of telson thus appears to be a good generic character.

References to the early descriptions of unidentified Porcellanid larvae are here omitted, but Hesse (1884) has described the larva of what he regarded as *Porcellana platycheles* from the stomach of a mackerel. It appears from his description and figures that this is clearly *P. longicornis*.

The Larvae of the Genus *PORCELLANA*

Of this genus we know the pre-zoea, stage I and stage II and post-larva of *Porcellana longicornis* (see Sars (1889) and the present work), *P. platycheles* (the present work) and *P. inaequalis* (pre-zoea and larvae only) from the Red Sea (see Gurney, 1938*a*). Menon (1937, 1940) describes what he believes to be the larva and post-larva of *P. serratifrons* from India. Other larvae ascribed by him provisionally to *Petrolisthes* probably belong to *Porcellana*.

All the larvae are transparent with red, orange or yellow pigment, usually on the rostrum, posterior carapace spines, region of the stomach, on the abdominal somites and telson and at the base of the maxillipedes. The pre-zoea swims actively for some hours. The larvae are very active and swim vertically, horizontally or sideways, usually front foremost, dorsal surface upwards. They swim up to the light and usually remain near the surface when healthy. The main food consists of debris, but they are capable of eating dead or moribund organisms and frequently devour their own brothers and sisters. Sometimes the empty long spines can be seen emerging at the anus. A specimen was seen to eat a moribund copepod. The contents of the gut in specimens from the plankton are entirely detritus with no visible remains of organisms. The post-larva feeds on detritus also, picking up small pieces from the stones with its chelae and putting them into its mouth, besides feeding by the filter on its third maxillipedes as is usual in the adult Porcellanids. The post-larvae of *P. longicornis* and of *P. platycheles* which were watched used the third maxillipedes alternately as described by Nicol (1932) or both together. The fifth legs were used to pick off any extraneous substance from the body which was kept scrupulously clean.

All the known larvae of *Porcellana* agree in having the telson with the fifth long seta outside the central prominence in stage I, and in stage II having a sixth seta situated on the central prominence. The third maxillipede in stage II may bear 4 setae on its exopod (*P. longicornis*, *P. platycheles*) or be unarmed (*P. inaequalis*, see Gurney, 1938*a*). According to Menon (1937, 1940) there are 6 setae in the larva attributed to *P. serratifrons*.

Porcellana longicornis (L.) (Figs. 3, 5–7, 8*a*, *b*, 9)

The earliest recorded female in berry occurred at the end of January (28. i. 38) in a *Chaetopterus* tube from the outside grounds. The eggs were nearly ready to hatch. The earliest larvae recorded in the plankton were at the end of February (24. ii. 38). In subsequent years they were later and they usually begin about March with a maximum from May to August and continue until October, rarely November. Early larvae were still in the inshore plankton in the middle of November 1941. The eggs when ready to hatch measure 0·48 × 0·40 mm. The pre-zoea is about 1·6 mm. in length, exclusive of the long spines on the antennae and telson. After swimming about for some hours at the surface nearest the light the pre-zoea moulted to the first larval stage, I*a*.

Stage Ia (Fig. 3*a*, *b*). This stage is distinctly smaller than Sars's first stage, the legs are not so well developed, but the posterior carapace spines are slightly longer. The newly moulted larva measures 4·5–4·8 mm. from spine to spine. Rostrum 2·9 mm., posterior carapace spines 1·1 mm., or rather more, body 1·6 mm. in length. Red pigment occurs on the rostrum, chiefly at the tip and in the centre so as to form bars, in the mouth region, on the sides of the abdominal somites, on the base of the first and second maxillipedes and on the tips of the posterior carapace spines. The region of the liver is usually greenish. There are fine spines on the rostrum; the posterior spines each have two conspicuous spines ventrally near the origin, the first the larger of the two, and small spines behind. This is an important difference in distinguishing the species from *Porcellana platycheles*, for in stage I of the latter the large spines are absent. The rudiments of the third maxillipedes are bilobed, but the legs are mere buds (Fig. 3*b*). The third seta of the telson (=the first long seta) is armed at the tip in a peculiar manner with large and conspicuous hook-like spines (Fig. 4*a*). The significance of these spines as a specific character was suggested by Dr Gurney (private communication) and is of importance, as here again we have a distinguishing character from *P. platycheles* in which the armature of the seta is quite different with long fine spines. *Petrolisthes armatus* has long fine spines at the ends of all the setae.

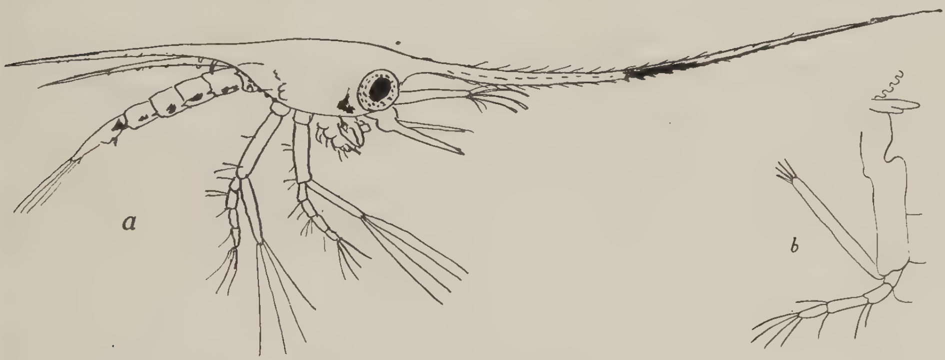

Fig. 3. *Porcellana longicornis*: *a*, stage I*a* from pre-zoea from egg, 4·6 mm. from spine to spine; *b*, second and third maxillipede and legs of same.

The pre-zoea in my experience has always moulted to I*a* and never to I*b*. I*a* has once moulted to II*a* which in all other cases came from I*b*. Thus *a* and *b* may be alternative stages. No moult has been observed from I*a* to I*b* and the way in which I*b* arises is consequently uncertain. The specimen of II*a* derived from I*a* was smaller than those from I*b* and this may account for the great variation in size in the later stages.

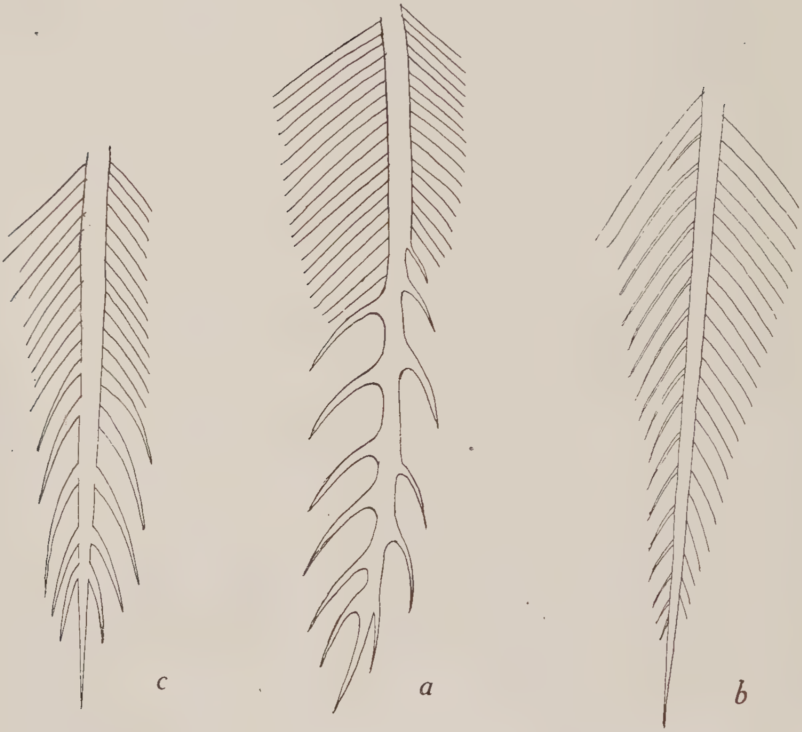

Fig. 4. Third seta of telson: *a*, *Porcellana longicornis*; *b*, *P. platycheles*; *c*, *Petrolisthes armatus*.

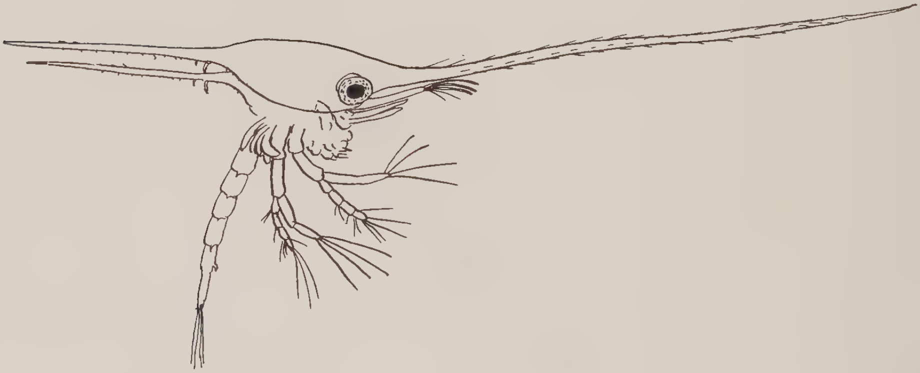

Fig. 5. *Porcellana longicornis*. Stage I*b*, from plankton, 5·8 mm. from spine to spine.

Stage Ib (Fig. 5). This stage agrees exactly with Sars's first stage and is the commonest stage of I in the plankton. The length of a typical specimen is 5·84 mm. from spine to spine, but the specimens may be longer or shorter. The rostrum is 3·3 mm.; the posterior carapace spines 1·5 mm; the length of the body, without rostrum, 2·2 mm. The form is the same as I*a* and the armature of the spines is the same, but the third maxillipede and legs are more developed. This stage from the plankton moulted into II*a* many times in finger bowls. It has never been seen to moult into II*b*.

Stage IIa (Figs. 6, 7 *a–f*). This stage is earlier than Sars's last stage, but of much the same form. It is frequently found in the plankton, and has moulted once from I*a* and many times from I*b* and itself moults to II*b*. The large ventral spines on the posterior carapace spines have now disappeared and only a few short spines remain. It has all the characters of stage II. The pleopods are very small and occur on somites 2–4. The telson has a sixth pair of long setae situated on the central prominence between the two short hairs. There are 11 setae on the exopods of the first and second maxillipedes and 4 on the exopod of the third maxillipede. The length of the long spines is very variable. A specimen which moulted from I*a* (4·5 mm. from spine to spine) measured 7·5 mm. from spine to spine. A typical specimen moulted from I*b* measured 8·8 mm. from spine to spine, the rostrum measured 5·6 mm., the posterior spines 2·5 mm., the body 2·6 mm. As a rule the posterior spines are about one-third the length of the rostrum, sometimes more, but never so much as half as they are in *Porcellana platycheles*. The legs are much more developed than they are in stage I, but still slender. Their lengths and the length of the pleopods are very variable. The endopod of the first leg shows signs of a chela, as also does the fifth leg which is hidden under the others. This stage moulted several times in finger bowls to stage II*b*. It is the stage figured by Gurney (1942, p. 256) as *Porcellana* sp.

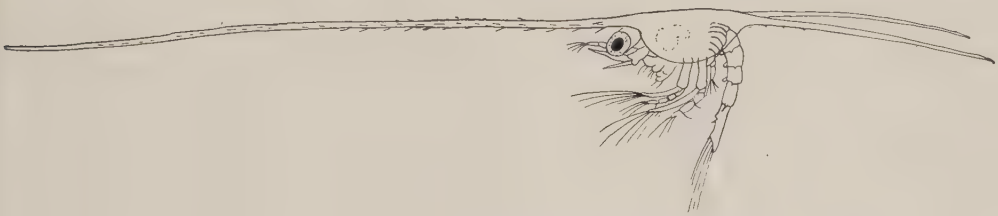

Fig. 6. *Porcellana longicornis*. Stage II*a* from I*b*, 9·3 mm. from spine to spine.

Stage II*b* (Fig. 7, *g–l*). This is similar to Sars's last stage and may be taken to be its equivalent although the third maxillipede and legs are rather longer. It differs little from II*a*, but the pleopods are distinctly longer and the third maxillipede and legs more developed. The rostrum and posterior spines vary much in length. A specimen may measure 9 mm. from spine to

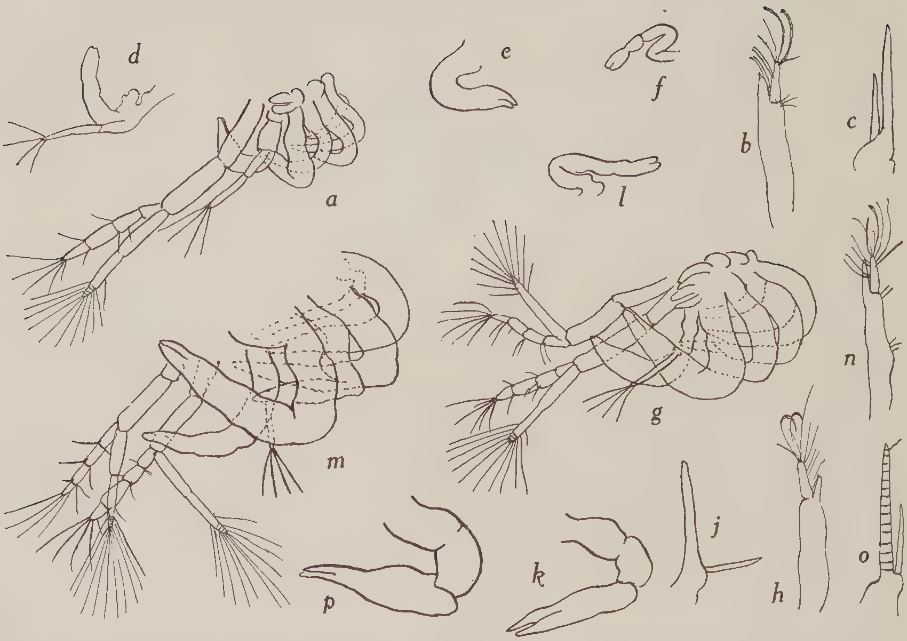

Fig. 7. *Porcellana longicornis*: *a*, stage II *a*, rather more developed than Fig. 6 from plankton; *b*, antennule; *c*, antenna; *d*, third maxillipede; *e*, first leg; *f*, fifth leg; *g*, stage II *b*; *h*, antennule; *j*, antenna; *k*, first leg; *l*, fifth leg; *m*, stage II *c* or late *b*; *n*, antennule; *o*, antenna; *p*, first leg.

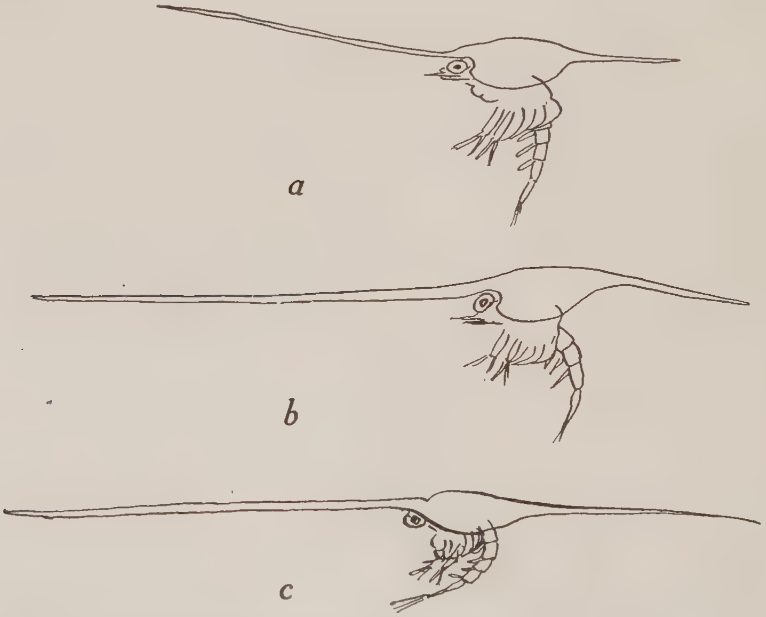

Fig. 8. *a*, *b*. *Porcellana longicornis*, last stage: *a*, with short spines; *b*, with long spines; *c*, *Porcellana platycheles*, last stage.

spine, but 11 mm. is not uncommon (Fig. 8). In some specimens the posterior spines are nearly double the length they are in others. In one measuring 9 mm. from spine to spine the rostrum is 5 mm.; posterior spine 2·5 mm.; body 3 mm. in length. Thus it is only the body and appendages which are larger than II*a*. This stage moulted several times to the post-larva, but there is a more advanced stage frequently found in the plankton which is here called II*c*. It has not been reared from II*b* nor has the post-larva been obtained from it. In lengths of spines and body it differs little from II*b*, but the third maxillipedes and legs are considerably larger (Fig. 7, *m–p*) making the thoracic region very heavy. The pleopods are larger and longer and the antennal flagellum is conspicuously jointed under the cuticle. It is possible that this is an alternative stage to II*b* or perhaps merely II*b* grown much more than usual before moulting to the post-larva, or it may be that a moult has taken place because conditions were unsuitable for an early change to post-larva.

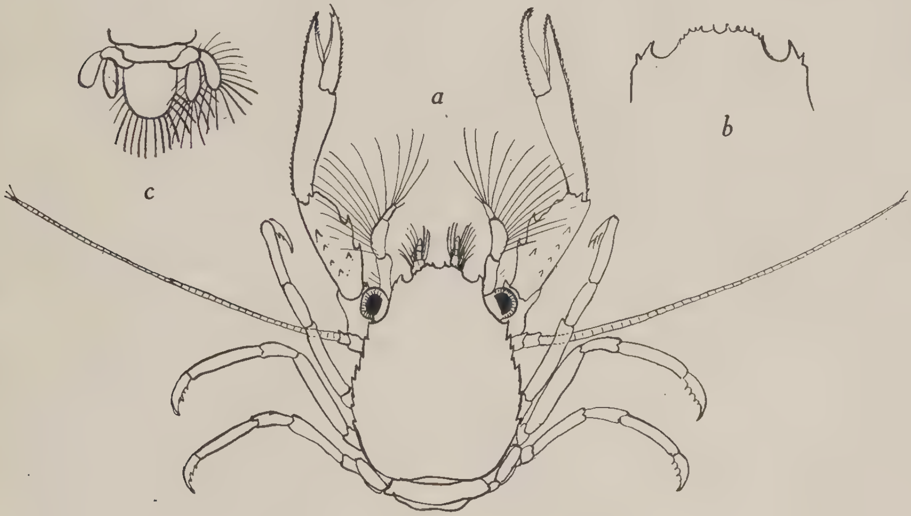

Fig. 9. *Porcellana longicornis*: *a*, post-larva from II*b*, carapace 1·6 mm. long; *b*, front of carapace; *c*, telson and uropods.

The Post-larva (Fig. 9 *a*, *b*, *c*). This was obtained several times by moult from II*b*. It is clear and transparent with small red chromatophores scattered about the body, especially in the front of the carapace and on the legs. Most of the chromatophores appear yellow by reflected light except those in the abdominal region which remain red. The post-larva can be recognized as a *Porcellana*, but is not very like the adult. The carapace measures 1·6 mm. in length and 1·3 mm. in breadth. The front is 3-lobed, the middle portion minutely toothed, whilst the lateral parts end in a strong tooth with small laterals. The sides of the carapace have strong teeth for nearly the whole length. The legs are functional, the chelae held out in front of the body when swimming, like a *Galathea*. The carpus of the chela is armed with 2 strong internal teeth, the propodus being fairly slender with hardly any hairs and bordered externally with small teeth. The dactyl has a similar armature. The dactyls of the remaining legs end in fairly strong claws and have 3 or 4 additional teeth. The sixth abdominal somite is now separated from the telson and bears setose uropods. There are 3 pairs of setose pleopods, on somites 2–4. The telson is not yet divided by sutures. It is rounded posteriorly and bordered by many setae. Although specimens lived for some time in bowls and plunger jars they all died before any moult was obtained.

The post-larval stage figured by Stebbing (1893) and attributed by him to this species is correctly identified, although Williamson (1915) regards it as probably belonging to *Porcellana platycheles*. Williamson has roughly described the larvae of *P. longicornis* and *P. platycheles*, but the details are insufficient to identify the two species accurately.

We thus have the following stages in the life history of *Porcellana longicornis*:

Egg hatches as a pre-zoea.
I*a* by moult from pre-zoea, also, rarely, in plankton.
II*b* in plankton only (not obtained by moult).
II*a* by moult from I*a* (once only) and I*b* (usually), also common in plankton.
II*b* by moult from II*a*, also common in plankton.
II*c* in plankton only (not obtained by moult).
Post-larva from II*b* by moult, also, rarely, in plankton.

Porcellana platycheles (Pennant) (Figs. 10, 11)

The adults are very common in the Plymouth district under stones between tide-marks on all rocky shores and down to about 3 fathoms. Breeding takes place from April to August, rarely earlier or later. No larvae have been seen after September. The larvae are present in the late spring and in the summer plankton inside the Breakwater, very rarely outside. The eggs are brownish green, measuring when ready to hatch 0·48–0·52 mm. in length and 0·42–0·45 mm. in breadth. The egg hatches as a pre-zoea measuring 2·5 mm. in length without the long spines. The rostral and posterior spines are folded in and much wrinkled (Fig. 1). There is red and orange pigment on the rostrum and spines and red and orange chromatophores show beneath the cuticle on the thorax and red on the abdominal somites. The antennule has long feathered spines, two at the tip and one on the side, the aesthetes of the first larva protruding slightly but not covered by spines. The antenna has 5 long feathered spines on the exopod and one on the endopod. The third maxillipede and legs are further developed than they are even in stage I*a* of *P. longicornis*.

The First Larva (stage I*b*, Fig. 10*a*). The first larva which moulted from the pre-zoea is in a later stage than in *P. longicornis*, stage I*a* being omitted. The rostral and posterior spines are very long and coloured red for nearly the whole length without bars. The red is, however, masked by a dull brown so that it rarely appears bright. The length from spine to spine in a typical specimen is 7·5–8 mm. In some of 8 mm. the rostrum measures 4·5 mm.; posterior spines 2·5 mm.; body 2·2 mm. The posterior spines are always half or more the length of the rostrum. The animal is clear and colourless except for a faintish pink tinge in the thorax, patches of red on the thorax and at the base of the maxillipedes, on the sides of the abdominal somites and base of telson. The rostrum curves upwards slightly and is covered with fine spines. The posterior spines reach a long way beyond the telson and have a few short spines, but no large spines such as distinguish the first stage of *P. longicornis*. The third telson spine (first long seta) has long fine spines, coarser however than on the main part, at its tip (Fig. 4*a*), in contrast to the hook-like spines of *P. longicornis*. Rudiments of the third maxillipede and legs are well advanced. This stage from the plankton moulted to the second stage similar to II*b* of *P. longicornis*, II*a* being omitted.

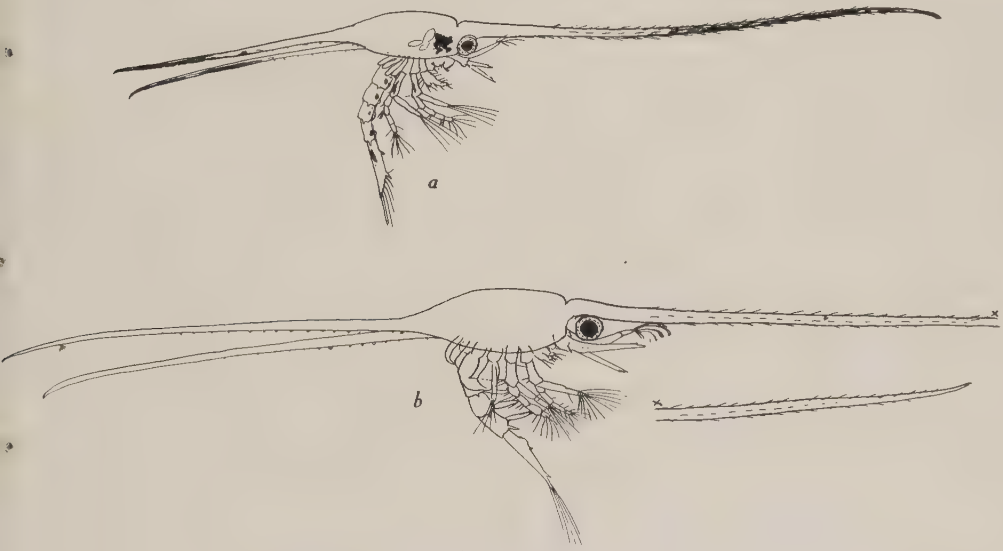

Fig. 10. *Porcellana platycheles*: *a*, stage I*b* from plankton, 8 mm. from spine to spine; *b*, stage II*b* from plankton, 11·5 mm. from spine to spine.

Stage IIb (Fig. 10*b*) moulted from I*b*, and was also obtained from the plankton. It is similar to II*b* of *P. longicornis*, but is variable in size. There are 4 pairs of pleopods, on somites 2–5, and these vary in length. In a specimen 11·5 mm. in length from spine to spine the rostrum was 7 mm.; posterior spine 4·2 mm.; body 3·5 mm. The telson has the sixth long seta situated on the central prominence as in all the species of *Porcellana*. There are 12 setae + a very small one on the exopods of the first and second maxillipedes and 4 on the third. There is a trace of the chela on the first leg and on the fifth leg and the legs are not very well developed. A specimen belonging to this stage from the plankton moulted to the post-larva in July and another one a few days afterwards. A third and a fourth died whilst moulting. There is a later stage similar to stage II*c* of *P. longicornis* in which the limbs are much more developed than in II*b*, but no moult was obtained from II*b*. One of these later stages died whilst moulting to the post-larva.

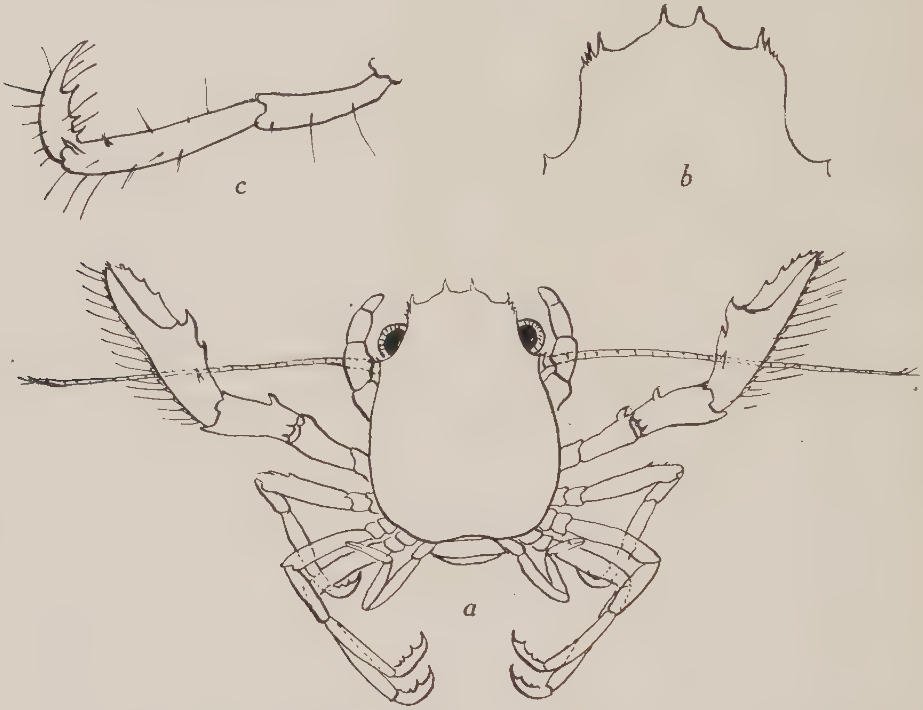

Fig. 11. *Porcellana platycheles*: *a*, post-larva from II*b*, 9·6 mm. across carapace; *b*, front of carapace; *c*, end of last leg.

The Post-larva (Fig. 11). This was obtained by moult from II*b* from the plankton. It is smaller than *P. longicornis* and the chelae are much more hairy and shorter. The telson is a simple plate. There are four pairs of setose pleopods and setose uropods. It is nearly colourless, but there are small scattered red chromatophores which become more conspicuous after the first day of moulting from the larva. No further stages were obtained. The carapace measured 1·44 mm. in length and 0·96 mm. in breadth. The front although roughly three-lobed projects more in the centre than in *P. longicornis* and has only 2 teeth, the lateral part having 4 teeth, the front one the largest. The third maxillipedes and first legs are shorter and broader than in *P. longicornis*, the first leg with only one internal tooth on the carpus and the propodus thick and stout with a fringe of thick hairs externally besides fine teeth. The dactylus is armed with a few strong teeth. The chelae in the two species foreshadow the form of the adult and it is easy to distinguish between them. The dactyls of the remaining legs have 3 teeth internal to the terminal claw.

The stages in the life history of *P. platycheles* may be summed up as follows:

Egg hatches as a pre-zoea.
I*b* by moult from pre-zoea.
II*b* from I*b* by moult.
II*c* in plankton (not obtained by moult).
Post-larva from II*b* and II*c* by moult.

P. platycheles thus has fewer stages than *P. longicornis*, I*a* and II*a* being omitted.

We are now in a position to compare the two species and the following table shows the chief differences:

	P. longicornis	*P. platycheles*
Number of larval stages	3 essential (2 alternative, making 5 in all) I*a*, I*b*, II*a*, II*b*, II*c*	2 essential (1 alternative, making 3 in all) I*b*, II*b*, II*c*
Posterior spines	about $\frac{1}{3}$ rostrum, never $\frac{1}{2}$	about $\frac{1}{2}$ rostrum, never less
Third telson spine	with hooks at end	with fine setae at end
Pleopods (stage II)	3 pairs on somites 2–4	4 pairs on somites 2–5
Armature of posterior spines (stage I)	2 conspicuous spines ventrally near the origin	these spines absent
Rostrum	red in wide bars	red, masked with brown evenly, not in bars

THE LARVAE OF THE GENUS *PETROLISTHES*

Petrolisthes armatus was hatched from the egg in Bermuda, but the late stages are as yet unknown. Gurney (1938*a*) describes an early and a late stage of a Porcellanid from the Red Sea which he attributes to this genus with a query. As from the reason he gives it is extremely likely that this is so, and the early larva agrees in essentials with that of *P. armatus*, we may assume that it really is a *Petrolisthes*. They differ from *Porcellana* in two points: (i) in the first larva the seventh seta of the telson (fifth long seta) arises from the central prominence, and (ii) (in the Red Sea form and, presumably, in *Petrolisthes armatus*) there is a central spine instead of an extra pair of setae in the late larva. The Red Sea form has 4 pairs of pleopods. The three larvae ascribed to *Petrolisthes* by Menon (1937) have all the characters of *Porcellana*, but, on the other hand, his *Porcellanella* larva agrees with *Petrolisthes* in essentials.

Petrolisthes armatus (Gibbes) (Fig. 12)

This species was hatched from the egg as a pre-zoea which swam about for some hours before moulting to the first larva. It was unfortunately impossible to be sure of the characters of the pre-zoea as the cuticle was so extremely delicate that it was always damaged.

The First Stage (I *a*). The rostrum and posterior spines are colourless, but there are patches of red chromatophores in the mouth region, on the base of the second maxillipedes, on the sides of the abdominal somites and dorsally on the telson. The length from spine to spine is 9 mm.; posterior spines 1·6 mm.; rostrum 6 mm.; body 2 mm. The rostrum is covered with small spines and minute protuberances. The posterior spines are covered with small spines which continue on to the posterior edge of the carapace. The telson is as broad as it is long. The appendages are very similar to those of *Porcellana*. The rudiments of the third maxillipedes and legs are exceedingly small. The long setae on the telson all have coarse spines at the tip, but the third seta (first long seta) has them larger and more numerous. No further stages of this species were obtained.

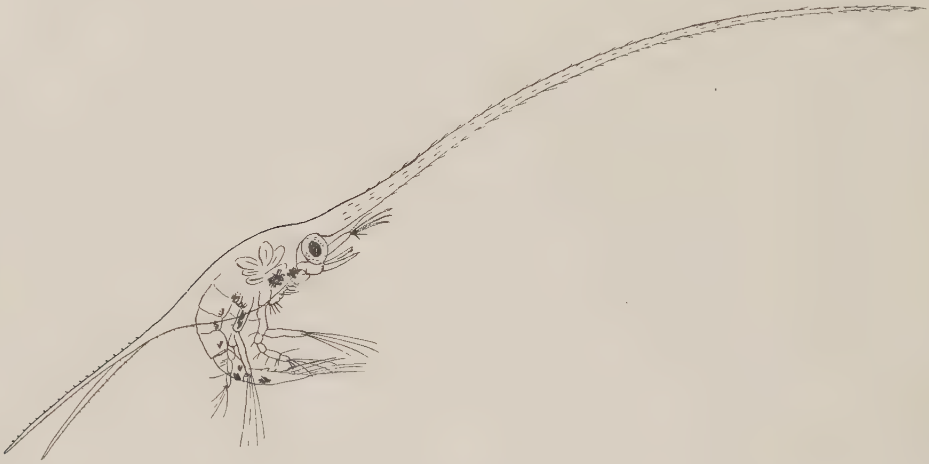

Fig. 12. *Petrolisthes armatus* from Bermuda. Stage I *a* from pre-zoea from egg, 9 mm. from spine to spine.

Gurney's (1938 *a*) description of stage II of *Petrolisthes* sp. shows that it differs little from stage II of *Porcellana* except in the telson as noted above. The exopod of the third maxillipede has no setae. He regards the species as having only two stages.

Faxon (1879) describes a larva which he attributes to *Polyonyx macrocheles* which resembles *Porcellana* in its telson and has pleopods on somites 2–5, thus resembling *P. platycheles*.

The known Porcellanid larvae thus fall into two groups divided by the form of telson:

A. Fifth long seta of telson situated outside the central prominence, a sixth seta on the prominence in stage II.

  I. Pleopods on abdominal somites 2, 3 and 4
  [*Porcellana longicornis*. Menon's *Petrolisthes* sp. 1 and 2, and his Porcellanid forms 1 and 2 (probably all *Porcellana*) (see Menon, 1937, 1940)]

  II. Pleopods on abdominal somites 2, 3, 4 and 5
  [*Porcellana platycheles*, *P. inaequalis*, Menon's Porcellanid, form 3 (=*P. serratifrons*?), Faxon's *Polyonyx macrocheles*]

B. Fifth long seta of telson on the central prominence, in late stages, no sixth seta, but a central tooth present

  Pleopods on abdominal somites 2, 3, 4 and 5 (so far as is known)
  [*Petrolisthes armatus*, only first larva known, so number of pleopods not ascertained, Gurney's *Petrolisthes* (?) sp., Menon's *Porcellanella* (probably *Petrolisthes*)]

LITERATURE

The references to the larvae will be found in Gurney's (1939) *Bibliography of the Larvae of Decapod Crustacea*, Ray Society, and (1942) *The Larvae of Decapod Crustacea*, Ray Society.

The following work is not included in the above:

Nicol, E. A. T., 1932. The feeding habits of the Galatheidae. *Journ. Mar. Biol. Assoc.*, Vol. xviii, pp. 87–106.

MEASUREMENT OF PHYTOPLANKTON POPULATION BY THE PIGMENT EXTRACTION METHOD

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Harvey's method of estimating concentration of planktonic algae was used by him to study seasonal abundance of net-caught algae in the waters around Plymouth (Harvey, 1934). His data showed that, in these circumstances, good agreement was to be expected between depth of tint of an acetone extract of algal pigments and the amount of algae, reckoned in terms of number multiplied by area or volume of individual cells (Harvey, 1935, p. 182). It does not necessarily follow, however, that agreement will be equally good in different circumstances. It is known, for instance, firstly, that the 'Harvey Standard' has not the same colour composition as acetone extracts from freshwater phytoplankton and, secondly, that extracts from phytoplankton collected at different seasons or from different localities are not of uniform colour (Riley, 1938, p. 371; Kozminski, 1938, p. 144). The colour of an acetone extract varies with the qualitative composition of the plankton; hence in the sea, as in fresh water, it is not to be expected that extracts from collections in which Bacillariophyceae (diatoms), Chrysophyceae (e.g. *Phaeocystis*, see Savage & Hardy, 1934) or Dinophyceae predominate will be of the same colour. It is the object of this paper, firstly, to specify two essential conditions which must be satisfied before the pigment-extraction method can estimate without avoidable error the concentrations of marine or freshwater phytoplankton and, secondly, both to indicate certain problems in need of further investigation and to suggest some lines of approach to these problems.

THE PIGMENTS IN PLANKTONIC ALGAE

Recent work by Kylin (1927), Seybold & Egle (1938), Carter, Heilbron & Lythgoe (1939) and Heilbron (1942) on the pigments present in the various classes of algae must be the starting point for a critical examination of the reliability of any modification of the Harvey method. The following composite table, abridged from the works referred to above, summarizes the chief differences between some classes of algae, members of which are likely to form the major part of a marine or freshwater plankton.

Seybold & Egle (1938) are of the opinion that chlorophyll B is completely absent from all classes of algae examined by them, save the Chlorophyceae. This view is not shared either by Kylin (1927) or by Dutton & Manning (1941, p. 521), and the latter authors prefer to write about the 'near absence' rather than the absence of chlorophyll B in the diatom *Nitzschia closterium*.

	Chlorophyll A	Chlorophyll B	'Xanthophyll'	Carotin	Fucoxanthin	Flavoxanthin	Phycoerythrin	{Phycoxanthin Myxoxanthophyll Aphanizophyll	Peridinin (Kylin, 1927)
CHLOROPHYCEAE	+	+	+	+	−	−	−	−	−
BACILLARIOPHYCEAE	+	−	+	+	+	−	−	−	−
CHRYSOPHYCEAE	+	−?	+	+	+	−	−	−	−
MYXOPHYCEAE	+	−	+	+	−	+	+	+	−
DINOPHYCEAE	+	−	+?	+	−	−	−	−	+

All workers are agreed, however, that in algae other than the Chlorophyceae the amount of chlorophyll B is very small indeed.

The pigments common to the classes of algae in the above table are chlorophyll A, 'xanthophyll' and carotin, and further work may show that these three are common to all aquatic algae. *The first essential condition, then, is that the estimate of algal abundance must be based on the concentration of one (or more) of these common pigments.*

THE SOLUBILITY OF ALGAL PIGMENTS IN ACETONE

If a mixed sample of freshwater algae be extracted for 12–24 hr. either with pure or 80% acetone in the cold, it is immediately apparent from examination under the microscope that only certain species have been completely decolorized. Virtually all diatoms will appear colourless; very many species of Chlorophyceae will apparently have lost but little of their original colour; certain of the Myxophyceae, including *Aphanizomenon*, *Anabaena* and *Microcystis* will be pale or dark blue; *Tribonema* (Xanthophyceae) and *Ceratium* (Dinophyceae) will be far from completely decolorized. Methyl alcohol is somewhat superior to acetone in its solvent action upon Chlorophyceae and Myxophyceae; it is inferior to acetone for diatoms. A mixture of equal parts of methyl alcohol and benzene has proved a good solvent. Pigments should be extracted as soon as possible after collection, for there is a loss of certain pigments when algae are dried in air (Seybold & Egle, 1938, p. 56). *The second essential condition, then, is that the solvent must extract completely the chlorophyll A, 'xanthophyll' or carotin from all members of a phytoplankton.*

THE CONSTANCY OF THE AMOUNTS OF THE COMMON PIGMENTS IN THE ALGAL CELL

In higher plants it is known, firstly, that 'the amount of chlorophyll A and B is not far from being constant, namely 0·6 to 1·4 grm. per 100 grm. of dried leaves', secondly, that 'the total weight of carotin and xanthophyll is from 0·07 to 0·2 grm. per 100 grm. of dried leaves, the ratio of carotin to xanthophyll being about 0·6' (Baly, 1940, p. 14). Excluding plants living in deep shade, it is probably not far from the truth to assert that the leaves of all terrestrial plants in this country receive light of the same spectral composition. In waters of widely differing colour and turbidity it is quite certain that the spectral composition of the subsurface light will not be constant (James & Birge, 1938, pp. 56–7; Cooper & Milne, 1938, p. 526). Stanbury (1931, p. 650) has shown that the colour of the diatom *Nitzschia closterium* varies from 'a rich dark brown shade to a decided greenish tint', when grown under light of different wave-length. Since the pigments normally present in diatoms are chlorophyll A, 'xanthophyll', carotin and fucoxanthin, it is, perhaps, legitimate to conclude by analogy from higher plants (Egle, 1937, p. 573) that any induced colour change will be the result of a shift in the ratios of the pigments. It follows, then, that in waters of widely differing colour and turbidity estimates of algal abundance based upon the extraction of one or more common pigments might be subject to considerable errors.

It has been suggested that the chlorophyll content not only varies with the physiological state of the algal cell, but also is not constant in different classes of algae (Riley, 1940, pp. 286–7). Chlorophyll separated by saponification with KOH, the method used by Riley, will be a mixture of chlorophylls A and B at those times when Chlorophyceae predominate; when other classes of algae are dominant it will be chlorophyll A only. The difference in the solvent action of acetone towards the several classes of algae must also be taken into account before the amounts actually present can be compared.

The reliability of the pigment-extraction method will depend to a great extent upon the constancy of the ratios of the common pigments both at different seasons and in different classes of algae, and it is imperative that measurements designed to put these points to the test shall be made. The selection of the common pigment should be influenced not only by the relative amount present in the different classes of algae, but also by the ease of separation (*vide infra*).

The Separation of Plant Pigments

Complete separation of algal pigments is possible by the Tswett chromatographic technique (Carter, Heilbron & Lythgoe, 1939; Cook, 1941; Zechmeister & Cholnoky, 1941). Of the various algal pigments, chlorophyll A is present in the greatest concentration, whence it follows that extracts from smaller quantities of algae would give more easily readable colours than would be the case if another pigment were selected as the common pigment. Owing to the ease with which carotin can be separated, however, the selection of carotin has much to recommend it. One of the real advantages of the Harvey method is the speed with which determinations can be made, and it is very desirable to retain this feature if possible. Methods for the separation of carotin in silage are modifications of the Tswett technique and details will be found in a recent paper by Bolton & Common (1942, p. 51). Should, however, chlorophyll A be selected, the standard for comparison must either be an artificial one or a solution of chlorophyll A, as used by Krey (1939, p. 205). Commercial chlorophyll being almost certainly prepared from higher plants (and hence a mixture of chlorophylls A and B) cannot be used as a standard except for Chlorophyceae.

GENERAL CONSIDERATIONS

There is insufficient evidence at the moment upon which to base an opinion as to the magnitude of the error likely to arise from seasonal or specific variations in the content of a common algal pigment. Gillam, El Ridi & Wimpenny (1939, p. 86), it is true, have stated that 'it seems unlikely that any arbitrary colorimetric measurement based on the amount of chlorophyll present in an acetone or ether suspension will give more than a rough estimate of the diatoms present in any sample', but it seems essential to obtain further data. If it may be assumed for the moment that the concentration of a common pigment is relatively constant in all classes of algae, there is little doubt that estimates based upon the complete extraction of one or more common pigments will tend to approach nearer the truth than will estimates expressed in terms of numbers multiplied by area or volume of individual cells. The difficulties of making such weighted counts are considerable; at seasons when Myxophyceae are dominant in a plankton they are very great. If it should turn out that wide differences are characteristic of the several classes of algae, it may be necessary to know the relative proportions of the different classes present. The chlorophyll B, fucoxanthin, 'phycocyanin' ratio would provide an approximate estimate of the proportions of Chlorophyceae, Bacillariophyceae plus Chrysophyceae and of Myxophyceae.

Although, admittedly, there are times, particularly in the spring, when agreement between weighted counts and depth of tint of an acetone extract is good, it is very unlikely that any modification of the Harvey method proposed to date, including that in use in these Laboratories, is sufficiently free from avoidable error to be more than an approximation. Hence, regional surveys in which chlorophyll is used as a measure of phytoplankton production (Deevey, 1940) may well give a misleading impression. It is essential not only to recognize the limitations of the method, as Riley has done (1939), but also to appreciate the factors, which in all probability, are responsible for a large part of these limitations. There is little doubt that certain sources of error can be eliminated and no time should be lost in seeking an improved technique. Unless this be done there is danger that much field work will later be found to have been of doubtful value.

My thanks are due to Lt.-Col. E. F. W. Mackenzie, O.B.E., M.C., Director of Water Examination, Metropolitan Water Board, for permission to submit this article for publication.

SUMMARY

1. In order that the Harvey method shall estimate without avoidable error the concentration of marine or freshwater phytoplankton, it is essential, firstly,

that the estimate be based upon the concentration of one or more pigments common to all classes of algae, secondly, that the solvent selected shall extract completely the common pigments.

2. Attention is directed to the possible influence of the spectral composition of subsurface light upon the pigment ratios in algal cells.

3. The use of carotin as the common pigment is suggested owing to the ease with which this pigment can be separated.

4. Failure to recognize the limitations of the method may materially reduce the value of certain types of field work.

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MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1941–42

The Council and the Officers.

During the year the Association has suffered a heavy loss in the death of Dr Guy Wood, who had been a Governor representing the Fishmongers' Company since 1931 and had been Honorary Treasurer for the past seven years. The Council also regret to note the death of Dr Cresswell Shearer, F.R.S., a Founder of the Association.

Major E. G. Christie-Miller has been nominated by the Fishmongers' Company to succeed Dr Guy Wood as a Governor, and at the meeting held in October he was elected Honorary Treasurer.

Four ordinary meetings of the Council were held during the year. Three of these were held in the rooms of the Royal Society and the thanks of the Association are due to the Society for this privilege. One meeting, in April, was held at Plymouth: it took place in the Grand Hotel since there were at the time unexploded bombs in and near the laboratory premises. The average attendance at the four meetings was twelve.

Air Raid Damage to the Plymouth Laboratory.

No additional damage of any consequence has been incurred during the year, though a few windows have been broken. The delayed action bombs which fell shortly before the April meeting were safely removed.

For the first three months of the year the staff of the Laboratory was largely engaged in repairing the damage occasioned in the March raids and though much will need to be done before the buildings are fully restored most of the laboratories have been made usable. Emergency repairs have been carried out in all the buildings: roofs have been made water-tight, and that of the specimen store has been renewed in corrugated iron; slating has been replaced, new rain-water gutters and pipes have been supplied and in nearly all the research rooms one window has been glazed. The heavily damaged southern end of the Easter class house has been removed and the northern part boarded in for use as a store, the roof of the constant temperature rooms has been boarded and a temporary roof has been put on the garage. The tank room cannot be restored until the end of the war: it has been closed throughout the year, thus depriving the Association of a valuable source of income.

Claims for compensation under the War Damage Act have been submitted on behalf of the Association. The claim under Part I of the Act (buildings) has been prepared by the Association's Architects and it is understood that, since the Laboratory comes under the heading of a Charity for the advancement of science or research, the amount of compensation will be determined by the War Damage Commission in consultation with representatives of the Association. No insurance premiums are payable under Part I of the Act. Under Part II (movable property) the Association is fully insured and a claim for property damaged or destroyed has been made.

The Council wishes to acknowledge with gratitude the receipt of a grant of £250 from a sum given by the American Philosophical Society to aid science and learning in Britain. The grant, which was made by the Royal Society, was given for emergency measures necessitated by recent air raid damage and essential for the maintenance of research work in the Laboratory.

Early in the year a house near Tavistock was rented by the Association. The house is used as accommodation for the Director, and for the storage of the Association's library. The Council decided at their April meeting that the library should be moved to a place of greater safety and it was transferred in May, certain reference books and recent volumes of some of the more important serials being retained at the Laboratory.

Plans for the rehabilitation of the Laboratory are being prepared, though it is evident that no work of this kind can be undertaken until the war is ended and that financial difficulties are likely to be considerable. The most urgent items will be (i) the renovation of the tank room, in order to restore the income derived from this source, (ii) the rebuilding of the south-eastern end of the North building, which was heavily damaged, and (iii) the provision of accommodation for the Easter class.

The Ship and Motor Boat.

The *Salpa* is still under requisition by the Admiralty and has not been available for the work of the Laboratory. The rate of hire for the ship has now been agreed with the Ministry of War Transport, the sums received from this source being credited to the *Salpa* Depreciation Fund. The motor boat *Gammarus* continues to work in restricted areas in Plymouth Sound and Cawsand Bay.

The Staff.

Since the date of the last Report the Association has temporarily lost the services of two more members of the scientific staff. Dr W. R. G. Atkins left the Laboratory on 7 July 1941, to take a commission in the Royal Army Medical Corps. Mr E. Ford has been given a commission in the Royal Air Force, in which he is assisting Mr F. S. Russell: he left on 20 November 1941. Seven members of the scientific staff are now away on National Service.

During the year the scientific staff of the Laboratory has been re-graded in accordance with a scheme recently approved for the Agricultural Research and Advisory Services. Under this scheme Dr W. R. G. Atkins and Mr E. Ford become Principal Scientific Officers, Mr F. S. Russell, Dr H. W. Harvey and

Dr M. V. Lebour become Senior Scientific Officers, while Dr A. Sand, Mr D. P. Wilson, Mr G. A. Steven, Mr G. M. Spooner and Dr L. H. N. Cooper are Scientific Officers.

The Association has lost the services of an old and highly valued member of the Laboratory staff during 1941. Captain V. Lord, who has been suffering from continued ill-health during the past two years, expressed the wish to retire from his post, and his resignation was accepted by the Council at their meeting in October. Captain Lord has been in the employment of the Association for twenty-eight and a half years, and all those who have worked with him have recognized his sterling qualities and the very valuable service he has rendered to the Laboratory.

Occupation of Tables.

The following have occupied tables at the Plymouth Laboratory during the year:

J. Cochemé (Self-retrieving spinner for mackerel).
P. G. Corbin (Plankton on mackerel grounds).
L. R. Crawshay, Plymouth (Sponges).
Dr Vera Fretter, London (Embryology of *Onchidella*).
A. T. Goodman (Library).
Dr M. Jepps, Glasgow (Life history of *Polystomella*).
A. G. Lowndes (Density of aquatic organisms).
Dr N. K. Panikkar, U.C., London (Osmoregulation in marine animals).
Dr M. W. Parke & Miss E. Clay (Algae).
W. J. Rees (Hydroids and Medusae).
Miss N. G. Sproston (Parasites of fishes).

Dr Paul Haas and Dr E. H. Thierry visited the Laboratory during the year in connexion with the investigations on marine algae.

No vacation courses were held during the year.

Special Research.

During the year there has been an increase in the work which the Laboratory has been asked to undertake on behalf of the Government. Advice has been given on a number of matters in reply to enquiries from service departments, and Dr Atkins, while at the Laboratory, continued to carry out confidential work on behalf of the Admiralty.

The curtailment of imported supplies of fish owing to war conditions has brought difficulties to a number of the colonies, and in several of them attempts to develop the local fisheries, some of them of considerable richness, are being made. Unfortunately information about these fisheries is most inadequate; their administration has been sadly neglected and even of the most important species of fish little or nothing is known of the life-history, growth-rate, migrations and other important factors. The Director of the Plymouth Laboratory has given some assistance to the Colonial Office in devising plans to meet war-time emergencies and it is hoped that the establishment of a number of suitably equipped fishery research stations will be included in post-war schemes for colonial development.

An extensive programme of work on marine algae has been put in hand during the year. Red algae are now in demand for the manufacture of bacteriological agar, while large quantities of brown algae are being used as raw material for national service requirements. Our knowledge of the biology of the brown algae is unfortunately scanty, and series of experiments have been begun at Wembury and on the west coast of Scotland. These experiments are being carried out by Dr Mary Parke, whose services were transferred to the Plymouth Laboratory at the beginning of the war, and others on the same lines are being conducted by Dr Margery Knight in the Isle of Man. Strips of beach have been cleared of weed in order to observe the new growth and many plants have been measured and marked, some being cut in order to follow the extent of regeneration.

The Association has also been asked to undertake surveys in south-west England, in Wales and in Scotland to ascertain the areas where brown algae are to be found in the greatest abundance. This work, which is beyond the resources of the greatly depleted laboratory staff, is being carried out for the Association by Dr V. J. Chapman and a party of research students from the School of Botany at Cambridge.

With one possible exception the red algae which can be employed in making agar occur in such small quantities that they cannot be used commercially. Dr A. P. Orr, of the Scottish Marine Biological Association, has taken a leading part in this work and experiments which he has made on the production of an agar substitute from other red algae have given very promising results. The Laboratory is co-operating in the developments which are now taking place and it is to be expected that satisfactory methods of producing culture media will shortly be evolved.

Tests on the durability under sea water of cords, textiles and netting, manufactured for service requirements, are now in progress. It would have been difficult or impossible to carry out these tests, which are on a far larger scale than any hitherto attempted by the Laboratory, but for the helpful assistance of Mr F. Payne, Manager of the Constructive Department, Devonport Dockyard, through whom the Association has obtained the loan of a large raft belonging to the Admiralty. Tests on the durability of the insulation in submarine cables are also being made and plates for the study of antifouling problems have been exposed.

Scientific Work of the Plymouth Laboratory Staff.

Interruptions due to air raids, and the absence of a large proportion of the scientific staff, have resulted in a greatly reduced output of scientific work.

H. W. Harvey has been engaged in investigating the phosphate set free from organic matter in solution in sea water by bacterial action and hydrolysis. These experiments were interrupted and the opportunity was taken to collate the results of published observations relating to factors which control the production of plant life in the sea. A review of recent developments in this field is being published in *Biological Reviews*.

Miss Lebour has published a paper in the *Annals and Magazine of Natural History* on the larval *Processa* of Bermuda, with a description of a new species, with which the Plymouth species are compared. The same paper deals with the larval Thalassinids of Bermuda. She has also published a joint paper with Dr R. Gurney on various larval decapods from Bermuda. Whenever possible she has continued her work on larval decapods and Mollusca from Plymouth; but she has been hampered by war-time restrictions in collecting and some of the material on which she was working was destroyed in air raids. Work on larval Porcellanids, crab larvae and *Teredo* has progressed, and tow-nettings from inside the Sound are examined regularly, the object being to ascertain the general contents of the hauls, and especially to identify the larvae in order to continue the study of life-histories of the decapod Crustacea and Mollusca and to add to our knowledge of the breeding seasons.

Mr D. P. Wilson has continued his observations and experiments on *Nitzschia closterium* in culture. There is now evidence that in addition to the formation of normal spindle-shaped cells from triradiate cells through an intermediate oval form without horns, there sometimes occurs in triradiate cultures a transformation into the spindle-shape by a direct elimination of one of the three horns. This appears to have taken place in a clone triradiate culture rather suddenly and on a large scale. Since the last report some triradiate cells have been obtained by the growth of hornless forms derived from normal spindle-shaped cells; change in this direction is much rarer than in the opposite. So far no consistent correlation between cell shape and the physical conditions under which growth has proceeded has been found, although it has appeared that light intensity or its duration may not be without effect. Experiments are still in progress.

Since last mentioned in these reports, considerable progress has been made in the study of the effect of winter tank temperatures on *Sepia officinalis* in captivity. The temperature of the aquarium water fluctuates with the air temperature and during frosty weather cuttle-fish are quickly killed. During the last two winters a small tank has been electrically heated and the temperature stabilized at about 13·5° C. In it a few cuttlefish have been kept in healthy condition much longer than before. When death took place in this tank it was found to be due to egg-binding in a solitary and fully mature female, or to injuries incurred during severe fighting between fully mature males at a time when females were not available. It seems probable that in order to keep *Sepia* in health for long periods under aquarium conditions, winter temperatures must be controlled and the sexes suitably balanced. A preliminary examination of *Sepia* shells collected over a period of years has indicated that most interesting results would be obtained by a more intensive study when material can again be obtained in quantity.

Mr Wilson has again assisted Mr J. Kingcome with the rearing of oysters in tanks at his fishery on the River Yealm. The summer of 1941 proved to be a very late season, due perhaps to the unusually cold weather in the spring when the parents were known to be very late in commencing shell growth. Eventually a small spat-fall was obtained. Last year's brood is growing well in cages in the estuary.

Since Mr Ford left, Mr Wilson has been sub-editor of the Journal, and he has also been engaged on plans for the restoration of the tank room.

Mrs Sexton has been engaged throughout the year with further detailed study of the genus *Gammarus*, particularly of the estuarine species and their relations with freshwater and purely marine forms. It is important to have the different species and their so-called varieties more strictly defined in view of their constant citation in ecological work. Particular attention has been paid to *Gammarus zaddachi* Sexton, a widespread species ranging from fresh to salt water, often confused with *G. locusta* (L.) at the seaward limit of its range, with *G. duebeni* Lillj. in the brackish water and with *G. pulex* in the freshwater. *G. zaddachi* develops an increase in the number of the sensory outgrowths of the cuticle in the freshwater environment, and at first glance, appears to be specifically different from the more spinose estuarine form; but the figures which have been prepared prove that structurally they are identical.

The Library.

As explained earlier in this Report the library has been moved out of Plymouth, only a small amount of recent literature being retained at the Laboratory. Accessions, as was to be expected, are less numerous than in times of peace, but current numbers of many periodicals have been received and some books have been added. The thanks of the Association are due to those institutions and authors who have presented books and papers.

For purposes of insurance under the War Damage Act the Council decided to obtain a professional valuation of the library. The work was carried out early in the year by Mr R. Ridgill Trout, and the total amount of the valuation, including stocks of back numbers of the Journal, is £15,750.

Published Memoirs.

Vol. XXV, No. 2 of the Journal of the Association was issued in November 1941.

The following papers, the outcome of work done at the Laboratory, have been published elsewhere than in the Journal of the Association:

FRETTER, VERA, 1941. An abnormal male *Sepia officinalis*. *Proc. Malacol. Soc.*, Vol. XXIV, pp. 154–5.

GURNEY, ROBERT & LEBOUR, MARIE V., 1941. On the larvae of certain Crustacea Macrura, mainly from Bermuda. *Journ. Linn. Soc. Zool.*, Vol. XLI, pp. 89–181.

LEBOUR, MARIE V., 1941. Notes on Thalassinid and Processid Larvae (Crustacea Decapoda) from Bermuda. *Ann. Mag. Nat. Hist.*, Ser. 11, Vol. VII, pp. 401–20.

Lowndes, A. G., 1941. Rapid determination of water in animals and plants. *Nature*, Vol. cxlviii, p. 79.

Panikkar, N. Kesava, 1941. Osmotic behaviour of the fairy shrimp *Chirocephalus diaphanus* Prevost. *Journ. Exp. Biol.*, Vol. xviii, pp. 110–14.

Panikkar, N. Kesava & Sproston, Nora G., 1941. Osmotic relations of some metazoan parasites. *Parasitology*, Vol. xxxiii, pp. 214–23.

Rees, W. J., 1941. The hydroid of the medusa *Cosmetira pilosella* Forbes. *Proc. Roy. Soc. Edinb.*, B, Vol. lxi, pp. 55–8.

Waterman, T. H., 1941. A comparative study of the effects of ions on whole nerve and isolated single nerve fibre preparations of crustacean neuromuscular systems. *Journ. Cell. Comp. Phys.*, Vol. xviii, pp. 109–26.

Wells, G. P., 1942. The action of potassium on echinoderm, molluscan and crustacean muscle. *Journ. Exp. Biol.*, Vol. xviii, pp. 213–22.

Wilson, D. P. & Lucas, C. E., 1942. *Nitzschia* cultures at Hull and at Plymouth. *Nature*, Vol. cxlix, p. 331.

Membership of the Association.

There have been no changes in the lists of Vice-Presidents and Associate Members. The total number of members of the Association on 31 March 1942 was 312 as compared with 313 at the corresponding date in 1941. During the year one life member was elected and two annual members compounded for their annual subscriptions.

Finance.

Grant from Development Fund. The Council have again to express their thanks to the Development Commissioners for their continued support of the general work of the Laboratory.

Private Income. The Council gratefully acknowledges the following generous grants for the year:

From the Fishmongers' Company (£600), the Royal Society (£50), Magdalen College, Oxford (£25), and the Cornwall Sea Fisheries Committee (£10). The following sums have also been received as rentals of tables in the Laboratory: The Universities of Cambridge (£105), London (£105), Oxford (£52. 10*s*.), Bristol (£25), Birmingham (£15. 15*s*.), Manchester (£10. 10*s*.), Leeds (£10. 10*s*.), Sheffield (£5); the British Association (£50), the Physiological Society (£30), and the Ray Lankester Fund (£20).

President, Vice-Presidents, Officers and Council.

The following is the list of those proposed by the Council for election for the year 1942–43.

President

G. P. BIDDER, SC.D.

Vice-Presidents

The Earl of STRADBROKE, K.C.M.G., C.B., C.V.O.
The Earl of IVEAGH, C.B., C.M.G.
Viscount ASTOR
Sir NICHOLAS WATERHOUSE, K.B.E.
The Lord MOYNE, P.C., D.S.O.
Sir SIDNEY HARMER, K.B.E., SC.D., F.R.S.
Sir P. CHALMERS MITCHELL, Kt., C.B.E., D.SC., F.R.S.
Col. E. T. PEEL, D.S.O., M.C.
Lord MILDMAY OF FLETE, P.C.
The Right Hon. Sir REGINALD DORMAN-SMITH, M.P.
Sir JOSEPH BARCROFT, C.B.E., F.R.S.
Prof. J. STANLEY GARDINER, F.R.S.
Prof. WALTER GARSTANG, D.SC.

COUNCIL

To retire in 1943

Prof. D. KEILIN, SC.D., F.R.S.
E. S. RUSSELL, O.B.E., D.SC.
Prof. E. J. SALISBURY, D.SC., F.R.S.
L. A. HARVEY
H. CARY GILSON

To retire in 1944

C. F. A. PANTIN, SC.D., F.R.S.
Prof. T. A. STEPHENSON, D.SC.
Prof. W. M. TATTERSALL, D.SC.
Prof. C. M. YONGE, D.SC.
J. Z. YOUNG

To retire in 1945

Prof. A. V. HILL, O.B.E., SC.D., Sec.R.S., M.P.
Prof. H. GRAHAM CANNON, SC.D., F.R.S.
The Hon. MIRIAM ROTHSCHILD
C. FORSTER-COOPER, M.A., SC.D., F.R.S.
Prof. A. C. HARDY, D.SC., F.R.S.

Chairman of Council

Prof. JAMES GRAY, M.C., SC.D., F.R.S.

Hon. Treasurer

Major E. G. CHRISTIE-MILLER, 71 Park Street, London, W. 1

Secretary

STANLEY KEMP, SC.D., F.R.S., The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

G. P. BIDDER, SC.D.
The Lord MOYNE, P.C., D.S.O.
A. T. A. DOBSON, C.B., C.V.O., C.B.E. (Ministry of Agriculture & Fisheries)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Admiral Sir AUBREY C. H. SMITH, K.B.E., C.B., M.V.O.
  Major E. G. CHRISTIE-MILLER
Prof. E. S. GOODRICH, D.SC., F.R.S. (Oxford University)
Prof. J. GRAY, M.C., SC.D., F.R.S. (Cambridge University)
Sir P. CHALMERS MITCHELL, Kt., C.B.E., D.SC., F.R.S. (British Association)
H. G. MAURICE, C.B. (Zoological Society)
Sir SIDNEY HARMER, K.B.E., SC.D., F.R.S. (Royal Society)
E. J. ALLEN, C.B.E., D.SC., F.R.S. (*Honorary*)

BALANCE SHEET 1941–42

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31ST MARCH 1942

	£	s.	d.	£	s.	d.	£	s.	d.
SUNDRY CREDITORS:									
Re-instatement of War Damage				272	19	1			
Staff Tax Deductions due to Inland Revenue ...				240	12	4			
Accrued Expenses				80	6	11			
Subscriptions received in advance				18	16	0			
Grant received in advance				150	0	0			
							762	14	4
INCOME TAX SUSPENSE ACCOUNT:									
As at 31st March 1941							53	9	8
AQUARIUM GUIDE PRINTING FUND:									
As at 31st March 1941							22	1	6
SPECIAL APPARATUS FUND:									
As at 31st March 1941				55	4	11			
Less: Expenditure				45	0	0			
							10	4	11
MACKEREL RESEARCH FUND:									
As at 31st March 1941				56	9	8			
Less: Expenditure				16	1	3			
							40	8	5
ALGAL RESOURCES SURVEY FUND:	£	s.	d.						
Grant Received				800	0	0			
Less: Expenditure	582	13	4						
Remuneration credited to Income and Expenditure Account	29	2	8						
				611	16	0			
							188	4	0
E. T. BROWNE BEQUESTS FUNDS:									
Building Fund, as at 31st March 1941	1050	8	5						
Interest on Investment	32	1	2						
				1082	9	7			
Library Fund, as at 31st March 1941	1030	19	7						
Interest on Investment	31	9	4						
				1062	8	11			
Special Apparatus Fund, as at 31st March 1941	2110	4	5						
Interest on Investment	64	8	0						
				2174	12	5			
Scientific Publications Fund, as at 31st March 1941	1582	13	3						
Interest on Investment	48	6	0						
				1630	19	3			
							5950	10	2

	£	s.	d.	£	s.	d.
BOATS AND EQUIPMENT, at valuation as estimated by the Director as at 31st March 1941:						
S/S "Salpa"	2000	0	0			
Motor Boat "Gammarus"	100	0	0			
Nets, Gear and General Equipment	30	0	0			
				2130	0	0
LABORATORY APPARATUS, ENGINES AND PUMPS, at valuation as estimated by the Director as at 31st March 1941				4000	0	0
LIBRARY, at valuation of Mr Ridgill Trout in January 1941				15750	0	0
STOCKS ON HAND, as valued by the Director:						
Specimens	1050	0	0			
Chemicals	150	0	0			
Journals	400	0	0			
				1600	0	0
SUNDRY DEBTORS:						
Sales of Specimens and Journals	217	17	6			
Sundries	126	13	6			
Income Tax Recoverable	54	13	4			
				399	4	4
PREPAYMENTS				48	2	0
GENERAL FUND INVESTMENT, at market value as at 31st March 1931:						
£352. 2*s.* 3*d.* Local Loans 3%				232	7	10
(Market value at date £339. 15*s.* 9*d.*)						
E. T. BROWNE BEQUEST FUNDS INVESTMENT, at cost:						
£6047. 9*s.* 10*d.* Conversion Loan 3%				5950	10	2
(Market value at date £6138. 4*s.* 1*d.*)						
"SALPA" DEPRECIATION FUND INVESTMENTS, at cost:						
£590. 6*s.* 0*d.* Local Loans 3%	506	10	9			
£3124. 9*s.* 1*d.* Conversion Loan 3%	3168	19	6			
(Market value at date £3740. 19*s.* 2*d.*)				3675	10	3
REPAIRS AND RENOVATION FUND INVESTMENT, at cost:						
£206. 12*s.* 1*d.* Conversion Loan 3%				208	10	7
(Market value at date £209. 14*s.* 1*d.*)						
COMPOSITION FEES FUND INVESTMENTS, at cost:						
£18. 8*s.* 6*d.* Local Loans 3%	15	15	0			
£200. 18*s.* [illegible]*d.* Conversion Loan 3%	[illegible]4	15	0			
(Market value at date £221. 14*s.* 9*d.*)						

“Salpa” Depreciation Fund:		
As at 31st March 1941	3062 11 9	
Add: Amount receivable from Ministry of War Transport on account of Hire	638 0 0	
Interest on Investments	91 18 6	
Less: Transfer to Repairs and Renovations Fund	50 0 0	
		3742 10 3
Repairs and Renovations Fund:		
As at 31st March 1941	258 4 3	
Add: Transfer from “Salpa” Depreciation Fund	50 0 0	
Interest on Investment	4 12 0	
		312 16 3
Composition Fees Fund:		
As at 31st March 1941	157 10 0	
Add: Fees Received	63 0 0	
		220 10 0
Capital Reserve Account:		
As at 31st March 1941		17311 8 2
Surplus:		
As at 31st March 1941	6872 19 10	
Add: Surplus for the year, as per Income and Expenditure Account	247 14 6	
		7120 14 4
		£35,735 12 0

	£ s. d.		
Cash at Bank and in Hand:			
Coutts & Company		1229 2 6	
Lloyds Bank Limited		141 4 8	
Cash in Hand		51 9 7	
			1421 16 9
Recoverable Expenditure:			
M. Parke Fund:			
As at 31st March 1941	110 19 2		
Expenditure	388 9 8		
	499 8 10		
Less: Grant Received	495 0 0		
		4 8 10	
Biological Investigations in Algae:			
Expenditure	504 9 9		
Less: Amounts Recovered ...	436 15 9		
		67 14 0	
Celanese Account:			
Expenditure		26 17 3	
			99 0 1
			£35,735 12 0

L. A. Harvey
T. A. Stephenson } *Members of Council.*

To the Members of the Marine Biological Association of the United Kingdom:

We report that we have examined the above Balance Sheet with the books of the Association and have obtained all the information and explanations we have required. Capital expenditure on erection of Buildings on Land held on Lease from the War Department is excluded. Subject to this remark we are of opinion that the Balance Sheet is properly drawn up so as to exhibit a true and correct view of the state of the Association's affairs as at 31st March 1942 according to the best of our information and the explanations given to us and as shown by the books of the Association.

Shinners Bridge, Totnes, S. Devon.
13*th May*, 1942.

Price, Waterhouse & Co.

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1942

	£	s.	d.	£	s.	d.
To Salaries, including the Association's Contributions to Superannuation				6505	18	8
,, Laboratory Wages, including National Insurance and the Association's Contributions to Superannuation				2496	17	6
,, Upkeep of Library				156	9	5
,, Scientific Publications, Less Sales				286	5	9
,, Upkeep of Laboratories and Tank Rooms:						
Buildings and Machinery	79	14	8			
Electricity, Gas, Coal, Oil and Water ...	399	18	6			
Chemicals and Apparatus	109	19	0			
Rates, Taxes and Insurance	137	7	6			
Travelling Expenses	53	11	4			
Stationery, Postages, Telephone, Carriage and Sundries	249	4	10			
Specimens	136	11	11			
				1166	7	9
,, Maintenance and Hire of Boats:						
Wages, including Diet Allowance, National Insurance and Casual Labour	874	1	0			
Coal, Water, Oil, Petrol, etc.	20	19	6			
Maintenance and Repairs, with Nets, Gear and Apparatus	16	1	7			
Purchase of Material for Nets for Sale, excluding Labour	63	19	1			
Boat Hire and Collecting Expenses	2	15	4			
Insurance	48	17	11			
				1026	14	5
,, Bank Charges				12	2	0
,, Buildings Extension Fund—Balance as at 31st March 1941 Written Off				54	3	2
,, War Time Expenditure:						
War Damage Contributions	418	3	3			
War Risk Insurance	78	15	0			
Accommodation for Director and Removal and Storage of Library	185	17	0			
Amounts payable to Firewatchers	60	5	0			
Re-instatement of War Damage to Buildings and Machinery (including Professional Fees)	477	5	2			
	1220	5	5			
Less: Grant received from American Philosophical Society and Sundry Donations	273	13	3			
				946	12	2
,, Balance, Being Surplus for the Year ...				247	14	6
				£12,899	5	4

	£	s.	d.	£	s.	d.
By Grants:						
Ministry of Agriculture and Fisheries Grant from Development Fund	10713	0	0			
Fishmongers' Company	600	0	0			
Royal Society	50	0	0			
British Association	50	0	0			
Physiological Society	60	0	0			
Cornwall Sea Fisheries Committee	10	0	0			
				11483	0	0
,, Subscriptions (excluding Subscriptions received in advance)				259	7	0
,, Donations				140	15	0
,, Sales:						
Specimens	526	10	9			
Fish	–	–	–			
Nets, Gear and Hydrographical Apparatus...	21	10	3			
				548	1	0
,, Table Rents (including University of Cambridge £105; Oxford £105; London £105; Bristol £25; Birmingham £15. 15*s.* 0*d.*; Leeds £10.10*s.*0*d.*; Manchester £10.10*s.*0*d.*; Sheffield £5; Imperial College £10; Trustees of Ray Lankester Fund £20)				422	5	0
,, Tank Room Receipts				–	–	–
,, Interest on Investments (Gross)				15	5	10
,, Sale of Dr M. V. Lebour's Book				1	0	10
,, Sale of "Plymouth Marine Fauna" ...					8	0
,, Remuneration in Connection with Algal Resources Survey				29	2	8
				£12,899	5	4

FEB 1957
LIBRARY
POFI
FISH & WILDLIFE
SERVICE